

SURGICAL STRATEGY IN PRIMARY PARATHYROID DISEASE

*with special reference to unilateral parathyroidectomy
and fat staining in the pathologic evaluation*

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*Life is meant to be lived,
not observed.*

The present dissertation is based on the following papers:

- I THE DIAGNOSTIC VALUE OF FAT STAINING IN PARATHYROID DISEASE AND ITS IMPACT ON SURGICAL STRATEGY: CLINICOPATHOLOGIC ANALYSIS OF 191 CASES

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- II UNILATERAL PARATHYROIDECTOMY IN HYPERPARATHYROIDISM DUE TO SINGLE ADENOMA

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- III SURGICAL STRATEGY IN HYPERPARATHYROIDISM DUE TO SOLITARY ADENOMA

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- IV ON THE SURGICAL STRATEGY IN NONFAMILIAL PRIMARY PARATHYROID HYPERPLASIA: LONG-TERM FOLLOW-UP OF 39 CASES

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- V CHRONIC PARATHYROIDITIS ASSOCIATED WITH PARATHYROID HYPERPLASIA AND HYPERPARATHYROIDISM

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These papers will be referred to in the text by their Roman numerals I - V.

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INTRODUCTION AND PURPOSE

In surgery of primary hyperparathyroidism (PHPT) the efficacy of intraoperative histopathology plays a central role for the choice of strategy. Because of the difficulty to distinguish between adenoma and hyperplasia with the routine stainings and partial biopsies commonly used, the opinions about what is proper surgical strategy are divergent, and extremes have evolved to eliminate the pathologic dilemma. Thus, removal of grossly enlarged gland(s) only is advocated by some (1,2) while subtotal parathyroidectomy in all patients is advocated by others (3). Undertreatment in cases of hyperplasia is a potential risk in the former category. In the latter one the majority of patients with adenoma are overtreated. Regardless of philosophy most authorities agree that identification of all four glands is a *sine qua non* in parathyroid surgery (4). In the light of new diagnostic principles (5) - i.e. fat staining of frozen sections and examination of two complete glands - this dogma can be seriously questioned. The value of methods making it possible to verify an adenoma without exploration of the contralateral side of the neck is obvious above all from safety point of view. However, what also makes this possibility most interesting is the facts that the vast majority of patients with PHPT has a solitary adenoma (6), and that efficient methods to localize a substantial proportion of these adenomas preoperatively are available (7).

In PHPT caused by a solitary adenoma cure is achieved by removal of the tumour-bearing gland. In PHPT caused by hyperplasia all glands are involved by the disease (6) and subtotal parathyroidectomy leaving no gland intact is in general recommended as the treatment of choice (4,8,9). In symptomatic patients with marked hypercalcemia and enlargement of all four glands this approach is unquestioned. It is less clear from the literature (10) if it is also the optimal approach in the increasing number of incidentally discovered asymptomatic patients with only slightly elevated serum calcium and mild degree of hyperplasia with one or more gland(s)



within normal range of size. The pathogenetic mechanism(s) behind primary parathyroid hyperplasia is still unknown. Knowledge in this field might help to individualize the treatment.

The present study was undertaken with the following objectives:

- To evaluate the efficacy of a modified oil-red-O fat staining technique for intraoperative distinction between adenoma and hyperplasia with two glands available (paper I).
- To evaluate a new surgical approach in PHPT utilizing fat staining and unilateral parathyroidectomy for intraoperative diagnosis with the aim to limit the operation to one side of the neck in patients with adenoma (papers II and III).
- To evaluate the long-term results of "conservative" surgery in patients with non-hereditary primary parathyroid hyperplasia where one or more normal-sized gland(s) were left intact (paper IV).
- To analyze the clinicopathologic picture in two cases of a previously unreported entity which might imply that immunologic mechanisms are involved in at least some cases of primary parathyroid hyperplasia (paper V).

MATERIAL AND METHODS

All patients in the present investigation were operated on for hyperparathyroidism at the Department of Surgery at Malmö General Hospital. None had been subjected to previous parathyroid surgery elsewhere.

Since 1977 fat staining of frozen sections has been used as a complement to conventional staining with hematoxylin-eosin to assess the functional activity of the parathyroid parenchyma. The two stainings were performed on consecutive sections. For intra-operative fat staining the tissue specimens were mounted unfixed in Tissue-Tek II O.C.T. Compound (Betlehem Trading Ltd.) and frozen by a jet of compressed CO_2 . Sections 4 to 6 microns thick were cut in a cryostat, mounted on glass slides and air dried. Lillie's supersaturated isopropanol method with oil-red-O (C.I. 26125) was applied in a modified way reducing the time consumption (11). The procedure was as follows:

1. A stock solution of saturated dye solution in 99 % isopropanol was prepared using 300 mg dye per 100 ml.
2. For staining 6 ml stock solution was diluted with 4 ml distilled water, filtered after 5 minutes and used immediately.
3. The sections were stained for 5 minutes and then thoroughly washed in tap water.
4. The sections were counterstained with Harris' or Ehrlich's hematoxylin for 15 to 30 seconds, blued in lithium carbonate solution (1 g in 120 ml distilled water), washed in tap water and mounted in Lillie's gum syrup.

Parathyroid chief cells were considered functionally inactive if they contained stainable intracytoplasmic fat droplets. Lack of such fat droplets was considered to indicate hyperfunction in a

state of hypercalcemia. These two principles were the basis of the diagnostic criteria applied for parathyroid adenoma and hyperplasia. These criteria are presented in detail below (see page 20).

I. To evaluate the diagnostic accuracy of fat staining, all cases of parathyroid surgery from 1977 to 1983 inclusive were reviewed. Included in the study were all cases fulfilling the following three prerequisites: 1) histologic examination of at least two complete parathyroid glands with 2) oil-red-O as well as hematoxylin-eosin, and 3) follow-up of serum calcium for at least one year if classified as adenoma. Altogether 191 cases fulfilled these criteria. The material comprised 165 cases of primary, 5 cases of secondary, and 21 cases of tertiary hyperparathyroidism. All histologic material was re-examined microscopically, and the medical records of the patients were reviewed for pertinent data.

II. Four groups, each consisting of 25 patients with PHPT due to solitary adenoma, were compared as to postoperative serum calcium changes, need for substitution treatment, operation time and late results with follow-up for at least two years. Non-parametric tests were used for the statistical analyses.

The patients were collected consecutively on the basis of the type of operation performed during a period of time partly preceding and partly succeeding the introduction of fat staining. In group one an adenoma and a normal gland were found at the first side explored. Both glands were removed in their entirety for histologic diagnosis including fat staining. The contralateral side was not explored. Group two comprised patients in whom two grossly normal glands were found at the first side explored. These were left intact. An adenoma and a normal fourth gland were found at the other side in which unilateral parathyroidectomy was performed for histologic diagnosis including fat staining. Group three

comprised patients who were explored bilaterally, and in whom extirpation of an adenoma and identification by partial biopsy of three normal glands were performed. The latter procedure was attempted at in the patients of group four but - besides removal of the adenoma - only one or two normal gland(s) were found and identified by partial biopsy.

III. This study was an evaluation of a consecutive series of 102 patients in whom unilateral parathyroidectomy was aimed at for diagnosis and treatment of an adenoma. The principle for the surgical method was to remove the presumed adenoma and one complete grossly normal gland, if possible from the same side of the neck - i.e. as a unilateral parathyroidectomy - for histologic diagnosis including fat staining. If two grossly normal glands were found on the first side these were left without biopsy and the contralateral side of the neck was explored. If an adenomatous and a grossly normal gland were found on the other side both were removed in their entirety for histologic diagnosis including fat staining. If no more than one grossly normal gland was found besides the presumed adenoma at bilateral exploration, only an incisional biopsy was taken from the normal-sized gland (in order to confirm the type of tissue). A normal-looking gland was not removed or biopsied until an abnormal gland had been found.

The material thus consisted of three groups of patients. In one group comprising 43 patients unilateral exploration and parathyroidectomy had been performed. In the second group comprising 45 patients bilateral exploration with unilateral parathyroidectomy had been performed. In the third group comprising 14 patients neither one of the aforementioned procedures could be accomplished. Comparisons were made as to changes in serum calcium during the first five postoperative days and the late results. All patients were followed for at least one year. Non-parametric tests were used for statistical analyses.

IV. To evaluate the long-term results of "conservative" surgery in non-hereditary PHPT caused by hyperplasia, the histopathologic records of all surgical specimens registered as any kind of parathyroid lesion from 1960 to 1980 inclusive were reviewed. The microscopic slides were re-examined in all cases with descriptions indicating that at least two completely removed glands were abnormal. When only hematoxylin-eosin stained sections were available an unequivocal enlargement of two glands were required as a criterion for hyperplasia besides compact and nodular histologic structure. Enlargement of a gland was defined as a weight exceeding 70 mg or - if weight was not available - a section area larger than 50 mm^2 on the microscopic slide. When fat stained sections were available smaller glands were accepted as hyperplastic if they contained compact areas or nodules of chief cells devoid of intracytoplasmic fat droplets indicating abnormal hyperactivity in a state of hypercalcemia.

Cases of secondary hyperparathyroidism were excluded by an evaluation of the renal function at the time of the operation. Patients with impaired renal function were accepted only if serum phosphate was low or normal. Patients with a history of familial parathyroid disease or hyperparathyroidism as part of a known multiple endocrine adenopathy syndrome were excluded.

Applying these criteria, 46 patients surgically treated for non-familial primary parathyroid hyperplasia were found. All were traced for follow-up, and the study was based on 39 of them in whom less than total parathyroidectomy had been performed, and the post-operative observation time as to serum calcium was at least two years. The surgical procedures performed were as follows:

No. of glands removed	No. of glands biopsied	No. of patients
2	-	7
2	1	2
3	-	17
2	2	7
3	1	6

Thus, the material could be divided into two major groups. In one group comprising 26 patients at least one gland was left intact without biopsy. In the second group comprising 13 patients none of four glands identified was completely spared irrespective of size. These two groups were compared as to late results.

V. At review of the histologic material in studies I and IV, two unique cases of PHPT were noticed. Their common denominator was hyperplasia with a marked chronic inflammation involving all glands examined (three in each case). At review of the literature in parathyroid disease this clinicopathologic picture appeared to be previously unreported, and an analysis with regard to possible pathogenetic mechanisms was made.

COMMENTS ON MATERIAL AND METHODS

The rationale for fat staining in PHPT is the electronmicroscopic observation that the content of lipid bodies in the parathyroid chief cell is inversely related to its content of organelles involved in hormone production, i.e. to its functional activity (12). Since the activity is related to the blood level of ionized calcium, functional suppression and consequently fat accumulation is to be expected in normal parathyroid parenchyma at hypercalcemia. Clinical application of this principle was first reported in 1976 by Roth and Gallagher (13).

The choice of fat dye and its solvent is of great importance in this context since the structures of interest are very small and thereby susceptible to the actually fat solvating fluids in which the dyes are dissolved. It should be pointed out that Herxheimer's mixture (equal parts of acetone and 70 % ethanol), which is a popular solvent for the commonly used Sudan dyes, contains only 15 % water and readily dissolves small fat droplets from the tissue (14). This problem is reduced by the weaker alcohol solution and 40 % water content of Lillie's isopropanol method employed in the present material. Furthermore, oil-red-O is considered to give better colour effects as well as more stable supersaturated solutions than other Sudan dyes (15). Methodologic differences probably are an important reason for the disagreement in the literature as to the practical value of fat staining in PHPT (16-20).

Good frozen sections as such are of course important to obtain useful information by the fat staining. The preparation of frozen sections largely is a handicraft with many variables involved that cannot be completely standardized. Due to this fact more sophisticated quantitative analyses of this material by means of morphometry have not been attempted. Anyway, the reliability of a subjective interpretation is the most relevant test of the efficacy of the method for intraoperative diagnosis. The fat stained frozen sections evaluated in the present study were prepared as part of

the routine work by any of the laboratory technicians on duty. With two technicians taking care of sectioning and staining respectively, the time consumption was ~~some five minutes extra~~ when oil-red-O was employed in addition to hematoxylin-eosin.

Consecutive frozen sections stained with hematoxylin-eosin and oil-red-O respectively are to be recommended for proper identification of the components in the tissue examined. Chief cells only, are of interest with regard to fat content for estimation of functional activity. Oxyphil cells must be excluded since they invariably show sparse fat content irrespective if they occur in normal, hyperplastic or adenomatous glands. Thymic tissue - which sometimes occur in close connection with the parathyroid glands - may mimic hyperactive parenchyma in fat stained material but is easily revealed to its nature with hematoxylin-eosin.

The reason for requiring **complete glands for differential diagnosis** between adenomatous and hyperplastic disease is the fact that hyperplasia may vary considerably in degree not only from one gland to another in the same patient but also within one and the same gland (6). Sometimes hyperplasia affects a gland only partially leaving areas that show a normal structure as well as an intraparenchymal fat content of the same magnitude as in normal glands in a patient with adenoma. Thus, a normal-looking partial biopsy never excludes hyperplastic disease since such a specimen not necessarily is representative for the gland as a whole.

The criteria for hyperplasia employed are based on the opinion that this disease always involves all of the parathyroid glands although it may affect them very asymmetrically and far from always appear as a "four gland disease" macroscopically (6,8). The choice of 70 mg as the upper limit of normal weight was based on recent autopsy studies (21,22). It is reasonable to assume that a gland with a section area of 50 mm^2 was heavier since the volume of such a specimen can be expected to have been more than 70 mm^3 , and 1 mm^3 roughly equals 1 mg of parathyroid tissue (23). Glands

weighing less than 70 mg were accepted as hyperplastic if fat stained sections were available and revealed compact areas or nodules of chief cells devoid of intracytoplasmic fat droplets indicating abnormal hyperactivity in a state of hypercalcemia. Nodules of oxyphil cells were not taken into account since such nodules may occur as an age related phenomenon in normal glands (6) and oxyphil cells normally have a sparse fat content. In each of the fat stained cases included in study IV at least two glands weighed more than 65 mg and all glands showed a nodular and compact structure as well as signs of hyperactivity. When considering the wide variation in size of normal glands (about 10 - 70 mg) it is obvious that a gland within normal limits of size may be hyperplastic - and histologically recognizable as such - if the process starts in a gland at the lower end of the normal variation. The rigour of the criteria for diagnosis of hyperplasia used in study IV is reflected by the fact that the selected cases represent only 11 % of all primary parathyroid operations performed during the sampling period. This rate of hyperplasia is equal to or less than in most reported series of PHPT (8,24).

An erroneous diagnosis of hyperplasia instead of **double adenomas** cannot be completely ruled out when only two glands are examined. Double adenomas are, however, extremely rare. When applying strict criteria for this diagnosis - i.e. demonstration of one complete, structurally as well as functionally normal gland besides two abnormal ones - it can be concluded from the recent fat stained material that the rate in Malmö is lower than 1 % of PHPT. This figure is based on the fact that only a single possible case has been recorded among some 200 patients in whom either a diagnosis of hyperplasia was based on access to three abnormal glands or a diagnosis of solitary adenoma was supported by follow-up for at least one year.

Patients with a history of **familial parathyroid disease** including patients with hyperparathyroidism as part of a multiple endocrine adenopathy syndrome were excluded from study IV because

of reported differences in clinical behavior in this category compared to patients with non-hereditary PHPT (25,26). The number of familial cases in the material was too low to warrant a separate analysis.

RESULTS AND COMMENTS

Fat staining for diagnosis in PHPT (I)

In 105 cases of PHPT an abnormal parathyroid gland was interpreted as adenoma on the basis of a normal second gland. By normal is here meant a microscopic picture consistent with functional inactivity (occurrence of fat droplets in the chief cells), lack of structurally divergent areas indicating secretory activity (absence of fat droplets in the chief cells), and a parenchymal cell mass estimated to be within normal limits as judged from the size of the gland and its relative content of parenchyma. The amount of stromal fat *per se* was not taken into account because of its marked variation with body constitution as well as age. Follow-up of serum calcium for at least one year (mean observation time 20 months) indicated that the adenoma diagnosis was correct in all patients but one. The reason for recurrent marginally elevated serum calcium after two months of postoperative normocalcemia in the latter patient is yet unknown, since her serum parathormone level is normal and she has not been reoperated. Unilateral neck exploration and parathyroidectomy was performed in 56 patients with adenoma diagnosis. In 41 of 49 bilaterally explored patients four glands were visualized.

Forty-two cases of PHPT were interpreted as hyperplasia because of distinct abnormalities in two glands. By abnormal is here meant lack of fat droplets (indicating secretory activity) in chief cells either diffusely or focally in nodules irrespective of the size of the gland. A third complete gland was available in 26 cases. All these additional glands were abnormal. The smallest gland observed in this series of hyperplasia measured 4x2x2 mm and was interpreted by the surgeon as normal. In this and another nine patients the gross findings on the first explored side of the neck were thought to represent an adenoma and a normal gland, while the microscopic picture clearly indicated hyperplasia. Consequently the other side was also explored, and additional grossly as well as microscopically abnormal glands were found in all 10 patients.

Difficulties in microscopic interpretation were encountered in 14 of the totally 165 cases of PHPT. In each of these patients two complete glands were removed, one of which was consistent with an adenoma and the other normal in size and ordinary in structure, but not unequivocally normal (i.e. functionally inactive) in fat stained sections. There was a generally sparse intraparenchymal fat content in some, while others showed markedly irregular distribution of intraparenchymal fat without any structurally divergent areas, or minute nodule-like chief cell aggregates free from fat. Glands from two very old patients showed prominent oxyphil cell nodules, which could have been age related changes. At follow-up one year after the operation all patients in this category were normocalcemic. The rate of equivocal findings with two glands available (probably adenoma but hyperplasia not excluded) being 8 % in this series of PHPT is sufficiently low for the method to be of value as a routine for limitation of surgery. It should be underlined that no harm is done by an undetermined intraoperative diagnosis at a unilateral exploration, since its only consequence is that the operation has to be continued and extended to a conventional bilateral one to arrive at a conclusion.

The results show that access to two complete parathyroid glands and use of fat staining with the modified oil-red-O method allow a highly reliable intraoperative distinction between adenoma and hyperplasia. A single possibly erroneous adenoma diagnosis makes a false "non-hyperplasia" rate of at most 1 % in this study. This high degree of diagnostic accuracy justifies limitation of surgery to one side of the neck when the prerequisites for an adenoma diagnosis as outlined above are fulfilled. In this context it should be pointed out that the consequences of a rare diagnostic error requiring reoperation are not as serious as after conventional exploration, since the reoperation can be limited to one, in advance known side where the tissue is virginal and surgery equal to a primary operation.

The findings in this fat stained material emphasize the importance of using complete glands to distinguish between adenomatous and hyperplastic disease. Several hyperplastic glands were considered by the surgeon to be normal but revealed distinct abnormalities microscopically. These abnormalities were, however, often focal. The structure could be normal with abundant stromal fat in one half of a gland, while the other half showed clearly abnormal nodular changes. Furthermore, not less than 50 % of the patients with primary hyperplasia had one or more gland(s) that partially showed an intraparenchymal fat content of the same magnitude as seen in functionally suppressed normal glands in patients with adenoma. Thus, it is apparent that a partial biopsy is insufficient to exclude hyperplasia, no matter how many glands that are biopsied. The loss of normal parathyroid tissue when one complete gland is removed hardly exceeds the loss when three glands are biopsied. Furthermore, the procedure recommended here not only provides the pathologist with a better basis for diagnosis, it also reduces the trauma to normal glands, leaving two intact when hyperplasia is excluded by one. The findings also emphasize the fact that adenoma and hyperplasia cannot be distinguished from each other with certainty by the microscopic picture of a single abnormal gland. An adenoma may be as multinodular as a hyperplastic gland, and a hyperplastic gland may have an adenomatous appearance, including presence of an extranodal rim of normal-looking parathyroid tissue. Not only can these disorders mimic each other structurally, but there is also an overlapping as to content and distribution of intraparenchymal fat droplets. Thus, two glands are needed for differential diagnosis also when fat staining is used.

Unilateral parathyroidectomy in PHPT due to adenoma (II, III)

The patients in study II can be divided into two major categories. First, those in whom the intraoperative diagnostic procedure was based on examination with fat staining of one complete grossly normal gland besides the presumed adenoma (group 1 and 2), secondly, those in whom the intraoperative diagnostic procedure was

based on the intention to identify all four glands (group 3 and 4). When comparing the length of surgery in these two categories it appeared that the operation time in the former category was on an average 25 % shorter than in the latter one ($p < 0.01$; Wilcoxon's rank sum test). Since the operations in both categories were performed or supervised by the same senior endocrine surgeon with only three other surgeons involved, it is reasonable to assume that this difference was related to the methods as such and not to the surgeons practicing them. There was no difference between the groups as to concomitant surgery of thyroid lesions. In this context it should be pointed out that reduction of the time of surgery also means reduction of exposure to anesthesia for the patient, which may be of particular value in the old age groups being quite common in parathyroid surgery.

When comparing early postoperative changes in serum calcium there was no significant difference in the rate (chi-square test) of transient hypocalcemia in unilaterally parathyroidectomized patients that had been explored on one and two sides respectively in study III. However, transient hypocalcemia lasted longer and showed lower values ($p < 0.01$ and $p < 0.05$ day 3 and 4 respectively; Mann-Whitney's rank sum test) in bilaterally explored patients compared to those with surgery limited to one side of the neck. This difference was more pronounced ($p < 0.01$; Wilcoxon's rank sum test) when comparing the unilaterally explored patients with those who were conventionally treated in study II, and hypocalcemia was also significantly more frequent in the latter category ($p < 0.01$; chi-square test). The unilaterally explored patients never dropped below 2.00 mmol/l in serum calcium (normal range 2.20 - 2.60). One might wonder why this category of patients developed hypocalcemia at all. One reasonable explanation is that the remaining normal but functionally suppressed glands needed some time to take up the hormone production required. There was no difference in the degree of preoperative hypercalcemia between the uni- and bilaterally explored groups, and there was no difference in serum alkaline phosphatase, which indicates that there was not more "bone hunger"

in the bilaterally explored and unilaterally parathyroidectomized patients that could explain their more protracted postoperative hypocalcemia compared to patients with surgery limited to one side of the neck. Neither could this difference be attributed to concomitant surgery of thyroid lesions. It is noteworthy that there was no difference as to rate and degree of transient hypocalcemia between bilaterally explored patients who underwent unilateral parathyroidectomy compared to those who had conventional surgery with biopsies from three normal glands (group 2 and 3 respectively in study II). This implies that the mere exploration of the parathyroid glands even in experienced hands may be equally traumatic as the biopsies. The loss of one whole normal gland did not induce more pronounced hypocalcemia than did partial biopsies from three normal glands.

At follow-up for a minimum of one year after surgery there was no recurrent disease among the unilaterally parathyroidectomized patients, and all of them were normocalcemic without substitution.

It should be mentioned that the patients in study II and III were operated on before any efficient method for preoperative localization of parathyroid adenomas was available at Malmö General Hospital. Thus, the chance to hit the adenoma side first was 50 % in this material. With the aid of various new imaging techniques at least two thirds of the adenomas can be localized preoperatively (7). Since half of the remaining adenomas still will be found by chance at the first side explored this means that limitation of surgery to one side of the neck is possible in some 80 % of the patients with adenoma if, for example, high-resolution ultrasonography is used.

"Conservative" surgery in non-hereditary PHPT due to hyperplasia (IV)

Out of 26 patients "conservatively" treated, with at least one gland left intact without biopsy, 22 were cured. Persisting hyper-

calcemia occurred in four patients, all of whom had three glands removed. Two of these four patients should not have been cured by "three and a half gland" resection or total parathyroidectomy with autotransplantation. One of these patients is still hypercalcemic after reoperation at which the latter procedure was performed. The other patient was cured after removal of an abnormally located supernumerary gland. Thus, two treatment failures in this group could be blamed on deliberate preservation of a gland considered to be normal-sized, which makes a failure rate of 8 %. At follow-up with an observation time of at least two years (median 4 years) no case of recurrent hyperparathyroidism after temporary postoperative normocalcemia was recorded.

Three more patients had been treated "conservatively" during the period covered by the study but were excluded because of follow-up shorter than two years. They all became normocalcemic after the operation and remained so as long as they were followed.

Among the 26 "conservatively" treated patients followed for at least two years, nine had less than four glands identified. Thus, the size of the gland(s) left intact is not known in these cases. In 17 patients, however, four glands were explored and the glands left intact without biopsy were considered to be normal-sized. The reason for avoiding biopsy was - besides the size of the glands - factors such as mild hypercalcemia, lack of symptoms, and advanced age or complicating diseases making a troublesome hypoparathyroidism especially unfortunate.

Among 13 more extensively treated patients - in whom no gland was left intact irrespective of size - there was no persisting hypercalcemia but two patients (15 %) developed permanent hypoparathyroidism requiring treatment with vitamin D.

A "conservative" approach, in the sense that only enlarged glands should be removed in PHPT due to hyperplasia, has been recommended by several authors in recent years (1,2,27). These authors stress,

however, that biopsies of grossly normal glands must be done. The results of the present study do not support that opinion. Biopsy from a grossly normal gland in a patient with established hyperplasia does not contribute to the diagnosis, but unquestionably increases the risk of inducing hypoparathyroidism (28). Thus, a partial biopsy should not be done but for identification of parathyroid tissue if the presence of a presumed gland is in doubt.

Chronic inflammation with hyperplasia in PHPT (V)

Both patients with this clinicopathologic picture were middle-aged men with kidney stones as presenting symptoms. Clinical investigation did not reveal any evidence of an underlying infectious disease or drug reaction that could explain the inflammatory component. Furthermore, the pathologic picture was found to be incompatible with any developmental anomaly such as incomplete separation from the thymus. Fat staining was of particular value in these cases since it clearly demonstrated that the parenchyma was not only hyperplastic but also hyperfunctioning, and thus that the hypercalcemia was not only caused by passive release of hormone from chief cells damaged by the inflammatory process. The clinico-pathologic triad comprising glandular hyperplasia, hyperfunction and lymphoid infiltration shows similarities to Graves' disease, which is associated with immunologic factors acting as stimulants of the thyroid gland. Unfortunately, the patients have not been accessible to any studies of possible immunologic abnormalities. Therefore, it cannot be proved but only suggested that an immunologic mechanism might have been involved in the pathogenesis of the parathyroid disease in these patients.

On the basis of this finding one may speculate if at least some patients with PHPT due to hyperplasia could be identified before operation by some type of serologic examination.

GENERAL SUMMARY AND CONCLUSIONS

The isopropanol oil-red-O method employed for fat staining in the present study was shown to be a most useful diagnostic aid in surgery of PHPT. With access to two complete glands this method - reflecting function in addition to structure of the glands - allows a highly reliable intraoperative distinction between adenomatous and hyperplastic disease. The diagnostically crucial specimen in a case presumed to represent an adenoma is not the enlarged gland but instead one of the associated glands, all of which shall be normal (i.e. functionally inactive in a state of hypercalcemia). If one gland is normal, hyperplasia is excluded since that is a process sparing no gland although the degree of involvement may vary from one gland to another in the same patient. Since hyperplastic changes may occur focally in a gland, a partial biopsy is insufficient to exclude hyperplasia, and nothing but a complete gland is useful for this purpose.

On the basis of the diagnostic accuracy achieved by fat staining as done here in conjunction with the use of complete glands for histologic examination, a new surgical strategy in PHPT is recommended. This strategy implies unilateral parathyroidectomy for diagnosis and treatment of an adenoma, with limitation of the operation to the tumour side if this is the first side to be explored and the diagnosis is verified by a second functionally normal (inactive) gland. If instead two grossly normal or two obviously abnormal glands are found, the other side is explored before any glands are removed. No normal-looking gland is removed until an abnormal one has been found, and the aim is to remove glands from the same side of the neck for diagnosis.

Unilateral exploration and parathyroidectomy in PHPT due to adenoma give, compared to conventional surgery (aiming at identification and biopsy of three normal glands), an equal cure rate, a significant gain in operation time, a significantly lower frequency and shorter duration of temporary postoperative hypocalcemia, as well

as total elimination of the risk for permanent hypoparathyroidism. The diagnostic and surgical methods described provide a basis for optimal utilization of ultrasonography and other facilities that allow preoperative localization of parathyroid adenomas.

The material indicates that in surgery of non-hereditary PHPT due to hyperplasia, subtotal parathyroidectomy leaving no gland intact is the method of choice only when all four glands are enlarged. If one or more gland(s) are grossly normal or near normal, factors such as degree of hypercalcemia, symptoms, age, general condition and life expectancy should be taken into consideration when deciding upon the extent of the operation. A more "conservative" surgery leaving at least one grossly normal gland intact without biopsy appears to be sufficient for cure in most of these cases, and minimizes the risk for development of permanent hypoparathyroidism.

On the basis of clinicopathologic findings in two patients it is suggested that immunologic mechanisms may be involved in the pathogenesis of at least some cases of "primary" parathyroid hyperplasia.

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*1. The diagnostic value of fat staining in parathyroid disease
and its impact on surgical strategy
Clinico-pathologic analysis of 191 cases*

A. Bondeson, L. Bondeson, O. Ljungberg, S. Tibblin

ABSTRACT

The study comprises 191 cases of surgically treated hyperparathyroidism with all principle types of parathyroid disease represented. At least two complete glands stained with a modified oil-red-O method for fat in addition to sections stained with hematoxylin-eosin were available in each case. On the basis of the morphologic evaluation and the clinical follow-up it is concluded that access to two complete glands and use of fat staining allow a highly reliable intraoperative distinction between adenoma and hyperplasia. Out of 105 cases with adenoma diagnosis followed for at least one year (mean 20 months) there was a single possible error. In each of 68 cases classified as hyperplasia on the basis of two abnormal glands every additional complete gland available (totally 182 glands) was at least partially abnormal with distinct signs of hyperactivity irrespective of size. The rate of equivocal findings with two glands available (probably adenoma but hyperplasia not excluded) was 8% in 165 cases of primary hyperparathyroidism. The results justify limitation of surgery to one side of the neck in cases with adenoma diagnosis based on access to a complete, functionally normal (inactive) gland besides the presumed adenoma. Thus, the methods described provide a basis for optimal utilization of ultrasonography and other facilities that allow preoperative localization of parathyroid adenomas.

INTRODUCTION

The role of fat staining in diagnosis of parathyroid disease is a controversial matter. According to some investigators fat staining provides no additional information over the usual surgical and pathological evaluation (1) and may even give contradictory information in many cases (2). Others consider this to be a reliable and valuable diagnostic aid in the essential intraoperative distinction between adenomatous and hyperplastic disease (3-5). Our experience conforms with the latter opinion, which as a practical consequence has resulted in a new surgical strategy in primary hyperparathyroidism (6,7). This is an analysis of our histologic findings correlated to clinical and biochemical data in 191 surgical cases including all principle types of parathyroid disease.

MATERIAL AND METHODS

The study comprises 191 patients operated on for hyperparathyroidism at the Department of Surgery at Malmö General Hospital from 1977 to 1983. None had been subjected to previous parathyroid surgery elsewhere. Each case included fulfills the following three prerequisites: 1) histologic examination of at least two complete glands with 2) modified oil-red-O (see below) as well as hematoxylin-eosin, and 3) follow-up for a minimum of one year if classified as adenoma. All microscopic slides were reviewed by two of the authors (AGB and LB). One of the authors (OL) was responsible for most of the original diagnostic reports.

The distribution of the material with regard to different types of parathyroid disease, age, sex and preoperative serum calcium is given in Table 1. The values of serum calcium given are the latest obtained before the operation. All samples were taken less than 24 hours preoperatively. Some patients showed normal values at that time but all had been hypercalcemic at several previous examinations with the exception of five patients with secondary hyperplasia. The group secondary hyperplasia comprises uremic patients with normocalcemia, while tertiary hyperparathyroidism is used to denote patients with secondary hyperparathyroidism and development of hypercalcemia.

Table I: Type of parathyroid disease, age and sex of the patient, and preoperative serum calcium* in 191 surgical cases of hyperparathyroidism. The figures do not give the frequency of the various entities in our material as a whole since this series is selected as described in the text.

Parathyroid disease	No	Sex		Age (years)		Preoperative S-Ca (mmol/l)**	
		♀	♂	Range	(Mean)	Range	(Mean)
Primary hyperparathyroidism	165	131	34	11-88	(64)	2.50-4.40	(2.82)
Adenoma	105	80	25	33-88	(65)	2.55-3.52	(2.82)
Chief cell hyperplasia	40	37	3	11-87	(62)	2.50-4.40	(2.78)
Equivocal cases*	14	10	4	17-88	(64)	2.63-3.50	(2.93)
Waterclear cell hyperplasia	2	2	-	37-78		2.68-3.25	
Hyperplastic parathyroiditis	2	-	2	53-62		2.55-2.75	
Lipoadenoma	1	1	-	72		3.00	
Carcinoma	1	1	-	64		2.72	
Secondary hyperplasia	5	4	1	15-55		2.15-2.50	
Tertiary hyperparathyroidism	21	12	9	17-77	(46)	2.63-3.73	(2.87)

*) See text for comment

**) Normal range: 2.20-2.60 mmol/l

The fat stain used was a modified oil-red-O method with a super-saturated isopropanol solution as described in detail elsewhere (8).

RESULTS

Adenoma

In all cases classified as adenoma we required a complete second gland with normal appearance. Any tissue available from a third and fourth gland also had to be normal. By normal is here meant a microscopic picture consistent with functional inactivity (occurrence of fat droplets in the chief cells), lack of structurally divergent areas indicating secretory activity (absence of fat droplets in the chief cells), and a parenchymal cell mass estimated to be within normal limits as judged from the size of the gland and its relative content of parenchyma. The amount of stromal fat *per se* was not taken into account because of its marked variation with body constitution as well as age.

The majority of the 105 adenomas showed no significant occurrence of intraparenchymal fat (i.e. fat droplets within the chief cells), although occasional minute droplets could be found in virtually all cases when the sections were scrutinized in high magnification (Fig 1). In 10 % of the adenomas, however, there was a noteworthy content of intraparenchymal fat. In half of these cases the distribution of the fat droplets was diffuse. The rest showed multifocally distributed droplets occurring either in streaks between nodular areas of fat-free parenchyma or as scattered accumulations without any special pattern. At comparison between the adenomas containing diffusely distributed fat droplets and their associated normal glandular tissue, the latter showed a contrasting and clearly more prominent intraparenchymal fat content with one exception. The patients with fat containing adenoma showed the same sex and age distribution as patients with adenoma in general and they also showed the same range of preoperative hypercalcemia. Preoperative phosphate treatment had been given in two cases which did not show any special characteristics. A normal parathyroid remnant was identified in fat stained frozen sections in 80 % of the adenoma bearing glands. There was in general a striking similarity between this remnant and the accompanying normal gland(s) with regard to the content of intraparenchymal fat droplets (Fig 1).

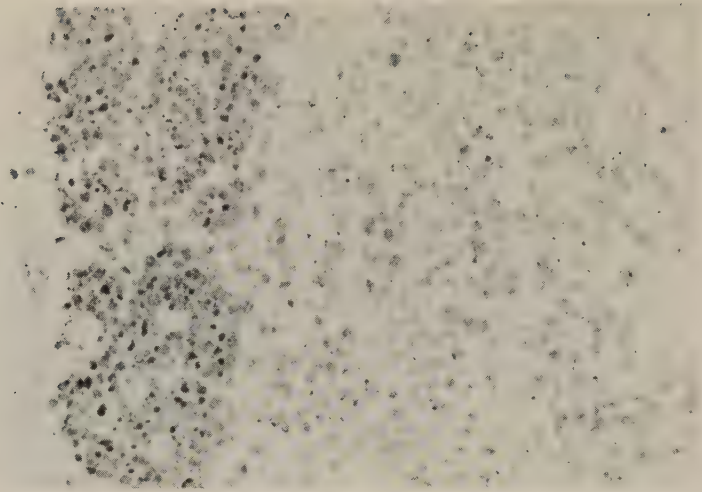


Fig 1. Adenoma to the right with a dark rim of normal parathyroid tissue to the left. Intraparenchymal fat is seen as black dots. Note their abundance in the functionally inactive normal tissue while only occasional minute droplets occur in the adenoma (oil-red-O, X200).

In the normal glands which were removed from patients with an adenoma there was a regular distribution of intraparenchymal fat on the whole, although fat droplets were not seen in every single chief cell. When the sections were scrutinized in high magnification minor irregularities of the fat droplet distribution comprising minute groups of chief cells were found in 20 % of these normal glands. Such inconspicuous irregularities were not associated with any structural divergence compared to the surrounding tissue such as nodule formation. Glands with questionable nodularity, sparse content of intraparenchymal fat on the whole, or conspicuous irregularities in its distribution despite normal structure have been classified as "equivocal cases" and are presented separately below. We found no apparent differences in intraparenchymal fat content with regard to sex, age or degree of preoperative hypercalcemia.

Four parathyroid glands were identified in 41 of the 105 patients with an adenoma. In another eight patients explored bilaterally

three glands were identified. In the remaining 56 patients a unilateral neck exploration and parathyroidectomy was performed.

All of the 105 patients with adenoma diagnosis but one were normocalcemic at the latest follow-up examination performed at least one year after the operation (mean observation time 20 months). The exception is a 65-year-old woman who underwent unilateral neck exploration and parathyroidectomy revealing a 310 mg adenomatous gland that microscopically showed a solitary chief cell nodule with a small rim of normal parathyroid tissue. The parenchyma of the latter was rich in fat droplets while the chief cell nodule was free from fat. The other gland was normal in size and structure with abundant and regularly distributed intraparenchymal fat droplets. Serum calcium fell from 2.75 to 2.20 mmol/l (normal 2.20-2.60 mmol/l) in the first day after the operation. She remained normocalcemic for two months but has later shown occasional, slightly elevated values (up to 2.65 mmol/l). If the cause is remaining diseased parathyroid glands is uncertain since selective venous samples from the neck show normal levels of parathormone, and the patient is not reoperated.

Hyperplasia

In cases classified as hyperplasia two microscopically abnormal glands were required. By abnormal is here meant lack of fat droplets in the chief cells either diffusely or focally in nodules. Cases with normal-sized gland(s) containing nodules composed entirely of oxyphil cells have been classified as "equivocal cases" since such nodules may occur as "normal" age related phenomena (9) as well as part of hyperplasia. Furthermore, oxyphil cells look the same at fat staining in normal and hyperplastic glands. In the 68 cases of hyperplasia 182 complete glands were examined including fat staining.

In the 40 patients with primary hyperplasia of chief cell type the intraparenchymal fat content showed a wide variation from one gland to another in the individual case as well as between different parts of one and the same gland. In half of the patients one or more gland(s) partially showed an intraparenchymal fat content of the same magnitude as in normal, functionally inactive glands accompanying an adenoma (Fig 2). It shall be stressed,

however, that although the fat-rich parenchyma could even dominate quantitatively all complete glands examined in cases of hyperplasia - irrespective of size - showed distinctly divergent, usually nodular, areas of parenchyma with very marked reduction or complete absence of fat droplets in the chief cells indicating secretory activity. In one third of the cases intraparenchymal fat droplets were relatively sparse and occurred in compressed streaks between dominating nodules free from fat (Fig 3). In seven cases (17 %) all glands examined were completely devoid of intraparenchymal fat. The patients in the fat-free group showed the same age range as the patients in the fat-rich one, and there was a broad overlapping between the two groups with regard to the degree of preoperative hypercalcemia. In each of the groups two patients had been given preoperative phosphate treatment. Two of the three patients with parathyroid hyperplasia as part of a multiple endocrine neoplasia syndrome belong to the fat-rich group and the third patient to the fat-free one.

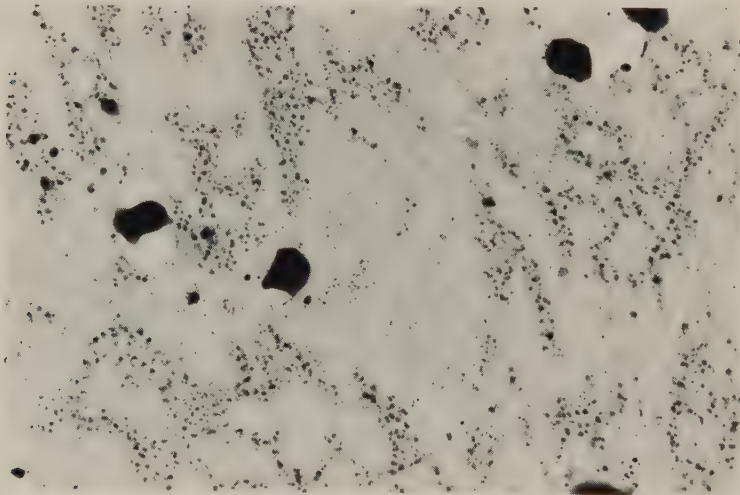


Fig 2. Hyperplastic gland with partially abundant intraparenchymal fat droplets appearing black (the few large black spots are stromal fat cells). Note the small but clearly abnormal chief cell nodule devoid of fat droplets indicating secretory activity in center (oil-red-O, X125).

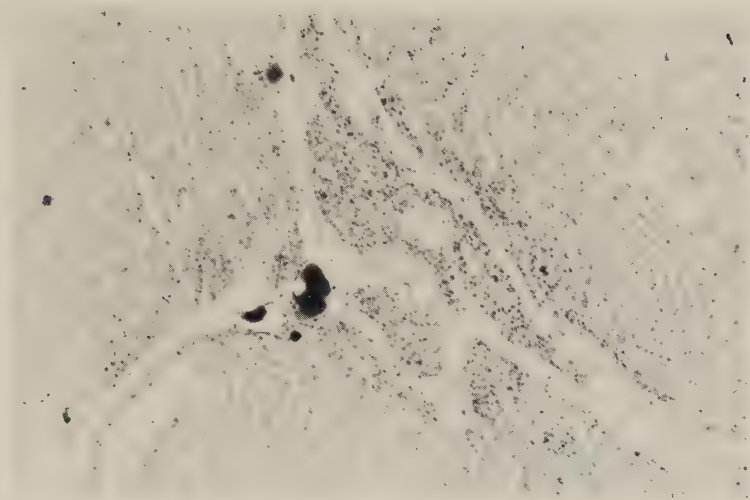


Fig 3. Hyperplastic gland with sparse intraparenchymal fat droplets occurring in dark compressed streaks between fat-free nodules (oil-red-O, X80).

The smallest gland observed in this series of hyperplasia measured only 4 x 2 x 2 mm and was interpreted by the surgeon as normal. In this and another nine patients the gross findings on the first explored side of the neck were thought to represent an adenoma and a normal gland, while the microscopic picture clearly indicated hyperplasia. Consequently the other side was also explored, and additional grossly as well as microscopically abnormal glands were found in all 10 patients.

In both patients with primary hyperplasia of waterclear cell type all glands removed were completely devoid of intraparenchymal fat.

All glands removed from patients with secondary hyperplasia were free from intraparenchymal fat droplets.

In cases of tertiary hyperparathyroidism the intraparenchymal fat content varied. Two thirds of the patients showed glands completely devoid of such fat. The minor group that showed varying amounts of intraparenchymal fat did not differ from the fat-free group with regard to the degree of preoperative hypercalcemia, and there were

no apparent differences related to preoperative duration of uremia or hemodialysis. Neither showed the glands from six patients with renal transplants any special characteristics. All complete glands examined were abnormal.

Rare lesions

In our single case of parathyroid carcinoma the tumor cells did not contain any fat. An accompanying normal gland showed abundant regularly distributed intraparenchymal fat droplets.

One case of parathyroid lipoadenoma was encountered (Fig 4). The parenchyma of the tumor contained only few fat droplets while a rim of normal parathyroid tissue on the tumorous gland as well as a second normal gland showed a most contrasting rich content of regularly distributed intraparenchymal fat.

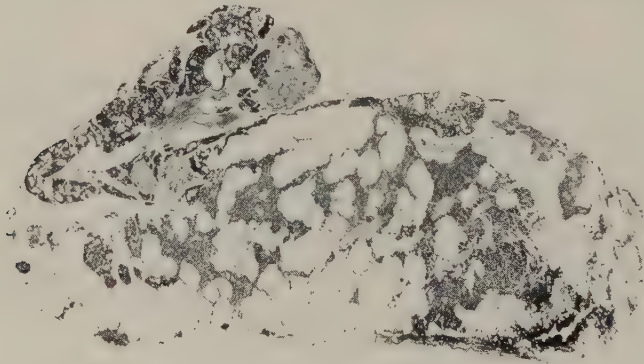


Fig 4. Lipoadenoma with dark remnant of normal glandular tissue to the upper left. The content of intraparenchymal fat is the reversed to the amount of stromal fat in the two components of this peculiar lesion (H&E, X6).

Our two cases of chronic parathyroiditis associated with parathyroid hyperplasia and hyperparathyroidism have been reported in detail elsewhere (10). All glands removed were abnormal and showed almost complete absence of intraparenchymal fat.

Equivocal cases

Difficulties in microscopic interpretation were encountered in 14 (8 %) of the totally 165 cases of primary hyperparathyroidism. In each of these patients two complete glands were removed, one of which was consistent with an adenoma and the other normal in size and ordinary in structure but not unequivocally normal (i.e. functionally inactive) in fat stained sections. These cases can be divided into four categories.

Firstly, a generally sparse intraparenchymal fat content in the normal-sized gland occurred in four patients. In all of these the adenomatous gland showed a rim of normally structured parathyroid tissue with the same sparse intraparenchymal fat content. Two patients were explored bilaterally. Two additional normal-sized glands were identified and left without biopsy in each of them. Three patients in this group were relatively young (17-44 years). The oldest patient was 77 years. Their preoperative serum calcium ranged from 2.63 to 3.10 mmol/l.

Secondly, a normal-sized gland with markedly irregular distribution of intraparenchymal fat without structurally divergent areas occurred in five patients. Two of the presumed adenomas had a multinodular structure with partially fat containing parenchyma. The other three were solitary nodules devoid of fat. In none of the five adenomatous glands any rim of normal glandular tissue was found. Two patients were explored bilaterally. Two additional normal-sized glands were identified and left without biopsy in each of them. In this group the age ranged from 66 to 81 years, and the preoperative serum calcium ranged from 2.75 to 3.20 mmol/l.

Thirdly, minute nodule-like chief cell aggregates free from fat in a normal-sized gland otherwise showing a picture consistent with inactivity occurred in three patients. In all of them the adenomatous gland showed a rim of normal parathyroid tissue with abundant intraparenchymal fat droplets. Two patients were explored bilaterally. In one of them no further glands were found, while an additional normal-sized gland was identified and left without biopsy in the other. The age of these patients ranged from 66 to 79 years, and their preoperative serum calcium ranged from 2.65 to 2.90 mmol/l.

Lastly, prominent oxyphil cell nodules in a normal-sized gland otherwise showing a picture consistent with inactivity occurred in two patients. Both had an adenomatous gland free from fat with a rim of normal parathyroid tissue showing abundant intraparenchymal fat. Structurally the oxyphil nodules were compatible with age related changes (the patients were 79 and 86 years of age).

All patients belonging to the group "equivocal cases" were normocalcemic at their last follow-up examination one year after the operation. None of them had received phosphate treatment preoperatively.

In this series we have not encountered any case fulfilling our criteria for double adenoma, i.e. demonstration of one complete, functionally normal (inactive) gland besides two abnormal glands.

DISCUSSION

The rationale for fat staining in this context is the ultrastructural observation that the fat content of the parathyroid chief cell is inversely related to its content of organelles involved in hormone production, i.e. its functional activity (11). Since the activity is related to the blood level of ionized calcium functional suppression and consequently fat accumulation of normal parathyroid parenchyma is to be expected in hypercalcemia. Thus, visualization of intraparenchymal fat would be of great value in the essential intraoperative distinction between "single gland" and "four gland" disease - i.e. adenoma and hyperplasia. However, from the first report on clinical application of this principle (3) its practical value has been debated, and quite divergent opinions have been expressed by other investigators (1,2, 4-8,12). Methodologic differences with regard to staining procedure and choice of fat dye as well as its solvent probably contribute to this disagreement.

Some investigators (1,2,12) have declared that fat staining does not provide any useful information over the usual surgical and pathological evaluation. On the basis of the material presented here - which is more than three times as large as any previously reported - we reject that statement.

There are a number of common macro- and microscopic constellations in which fat staining is a most useful diagnostic aid. One is the grossly normal gland showing a microscopically compact parenchyma with sparse stromal fat. Since the normal parenchymal cell mass may vary with a factor of not less than nine (13), and nothing is known about the size of the gland before it is taken out at surgery, it is not possible to tell from size alone if such a gland is normal or hyperplastic. Since the amount of stromal fat varies with age as well as body constitution and may be normally absent in young and/or lean individuals (Fig 5), lack of stromal fat cells as such cannot be taken as a reliable evidence of hyperplasia. In this situation it is obviously of great value to visualize the intra-parenchymal fat content, which reflects the activity of the gland.

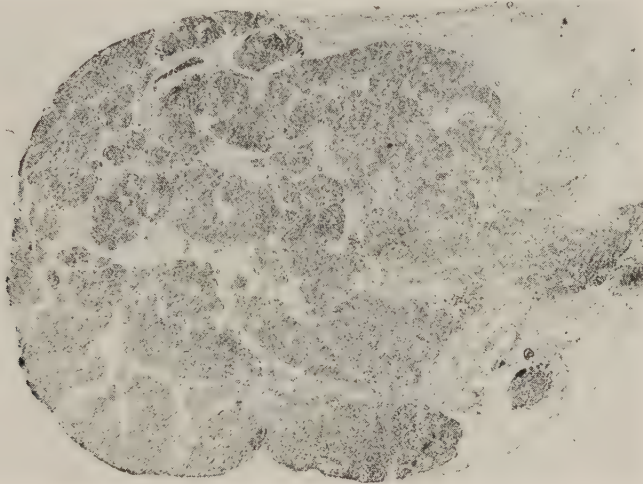


Fig 5. Normal gland from a lean 52-year-old patient with adenoma. Note the compact structure without stromal fat related to the body constitution of the patient. Hyperplasia was excluded by demonstration of abundant and regularly distributed intraparenchymal fat (H&E, X16)

Information about parenchymal activity obtained by fat staining also is of great value for interpretation of the grossly large gland showing much stromal fat and a parenchymal cell mass that is not obviously abnormal. The majority of normal glands weigh less than 50 mg but the normal range is wide with values skewed upwards

(13), and we have encountered normal glands with weights up to 90 mg explained by abundance of stromal fat (Fig 6). Early diffuse hyperplasia starting in that type of gland is difficult if at all possible to demonstrate microscopically without assessment of parenchymal activity. Lipoadenomas and the recently described peculiar entity named lipohyperplasia (14) are rare but potential diagnostic pitfalls that can be avoided by fat staining.

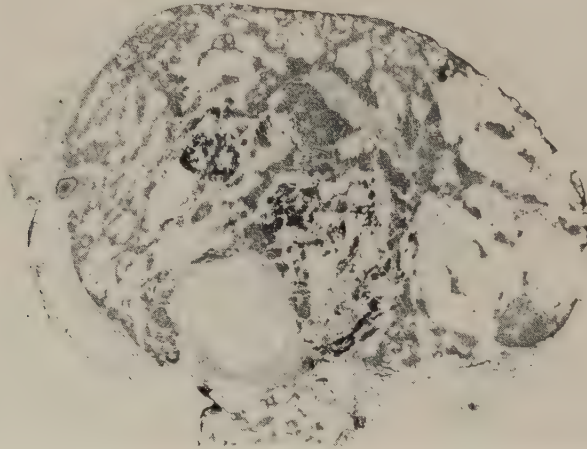


Fig 6. Large normal gland weighing 90 mg from an obese patient with adenoma. Hyperplasia was excluded by demonstration of abundant and regularly distributed intraparenchymal fat. Compare with figures 4 and 11 (H&E, X8).

In a parathyroid lesion presumed to represent an adenoma microscopic demonstration of a rim of normal glandular tissue provides valuable support for the adenoma diagnosis. It is our experience that this, usually tiny, component is much more easily seen in fat stained than in hematoxylin-eosin stained frozen sections, which has also been emphasized by other investigators (4,12). Because of its smallness it is often difficult and sometimes impossible for the pathologist to localize a normal parathyroid remnant on the adenoma by the naked eye. A practically useful fact is that the normal remnant in our experience almost always is to be found at the attachment of the vascular stalk (Fig 7). Therefore we mark the vascular stalk at the surgical dissection with a suture, which

facilitates the choice of material for frozen section examination. It shall be stressed, however, that an adenoma diagnosis cannot be based on a normal glandular remnant alone. Nodular hyperplasia occasionally may assume an indistinguishable picture (Fig 8), and thus examination of a second gland is essential.

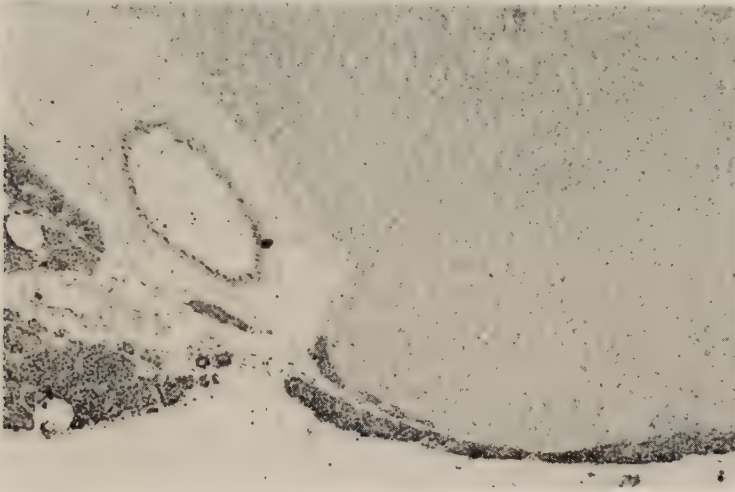


Fig 7. Adenoma with dark rim of normal glandular tissue at the lower left. Note the large vessel in this region. A normal parathyroid remnant is found at the attachment of the vascular stalk on most adenomas. A suture placed on the vessels at the surgical dissection facilitates the choice of optimal material for frozen sections (oil-red-O, X60).

A central question is what consequences an improved diagnostic accuracy achieved by fat staining may have for the surgical strategy in primary hyperparathyroidism. Can a presumed adenoma be verified and hyperplasia excluded without exploration of all four glands? In our experience this is definitely possible provided that a complete second gland is examined. Our opinion is based on the following.

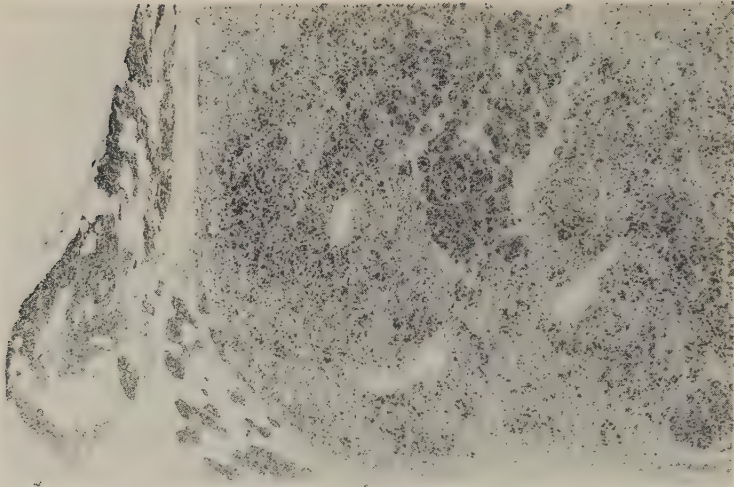


Fig 8. Hyperplastic gland structurally imitating an adenoma with rim of normal glandular tissue. The parathyroid hyperplasia in this case was part of a multiple endocrine neoplasia syndrome, and another two clearly abnormal glands were removed (H&E, X25).

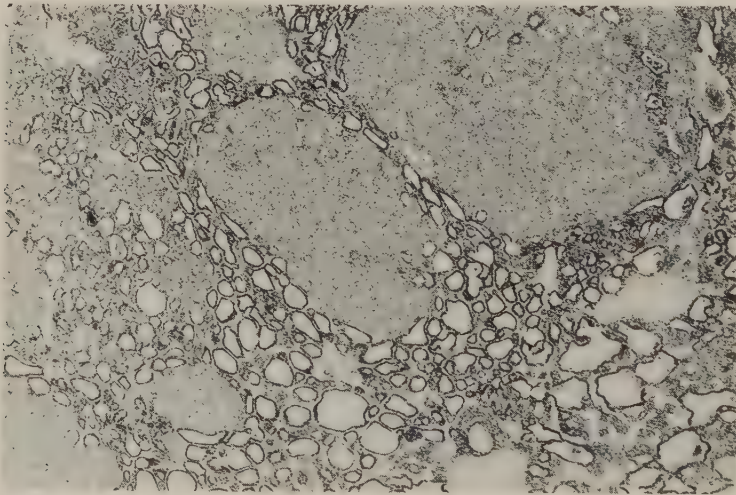


Fig 9. Adenoma with multinodular as well as follicular growth pattern illustrating the highly variable structure of this type of tumor. A complete second gland is essential for differential diagnosis adenoma versus hyperplasia (H&E, X25).

The adenoma may show a highly variable histologic structure and can be as multinodular as a hyperplastic gland (Fig 9). Conversely, hyperplasia may affect the four glands very asymmetrically giving the impression of a solitary adenoma. Hyperplasia may also imitate an adenoma histologically including presence of an extranodal rim of ordinary glandular tissue (Fig 8). Furthermore, there is overlapping between adenomas and hyperplastic glands with regard to content as well as distribution of intraparenchymal fat. From this it is apparent that a definite histologic diagnosis can hardly be made on only one gland irrespective if fat staining is used. In fact the diagnostically crucial specimen in a case presumed to represent an adenoma is not the enlarged gland but instead one of the associated glands, all of which shall be normal (with the very few exceptions of double adenomas). If one gland can be shown to be normal - i.e. inactive in a state of hypercalcemia - hyperplasia is excluded since that is a process involving all four glands (9). Here the fat stain reflecting activity is essential because of the poor correlation between structure and function of the parathyroid glands. It shall be emphasized that nothing but a complete gland is useful in this context. The reason for this is the fact that hyperplasia may affect a gland only partially (Fig 10 and 11) and leave areas showing a normal structure as well as intraparenchymal fat content of the same magnitude as in normal glands associated with an adenoma (Fig 2). Thus, the representativity of an incisional biopsy can always be questioned, and that type of specimen serves no other purpose than identification of parathyroid tissue as such.

A fundamental condition for justification of limited neck explorations in primary hyperparathyroidism is that signs of hyperactivity are indeed demonstrable to at least some extent in all four glands in every case of hyperplasia including glands within normal range of size and parenchymal cell mass. For natural reasons this has never been proven since some parathyroid tissue usually is left in the patient at surgery. We may conclude, however, that in each of our cases classified as hyperplasia on the basis of two abnormal glands every additional complete gland available was also abnormal with distinct signs of hyperactivity. Conversely, in all cases with one gland interpreted as normal all morphologic and clinical parameters including follow-up for a minimum of one year indicate that

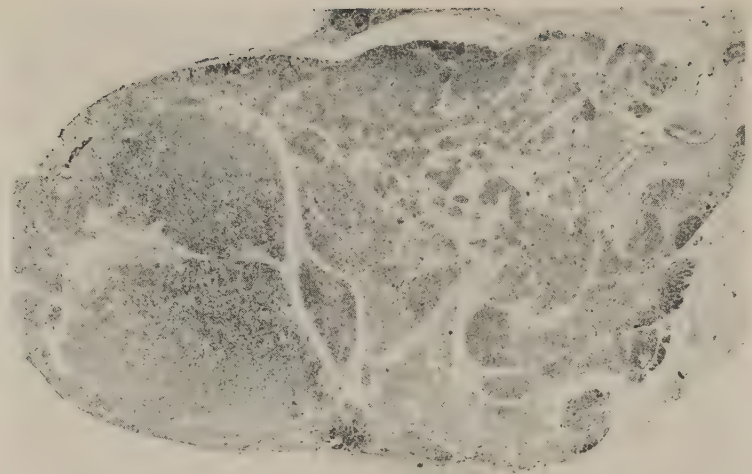


Fig 10. Hyperplasia with asymmetric involvement of a grossly near normal gland. Note the quite ordinary structure with abundant stromal fat in the right half contrasting to the solid nodules to the left. The structurally normal part also showed abundant intraparenchymal fat while the nodules were fat-free. Obviously a partial biopsy is insufficient to exclude hyperplasia and complete glands are essential for correct diagnosis (H&E, X12).

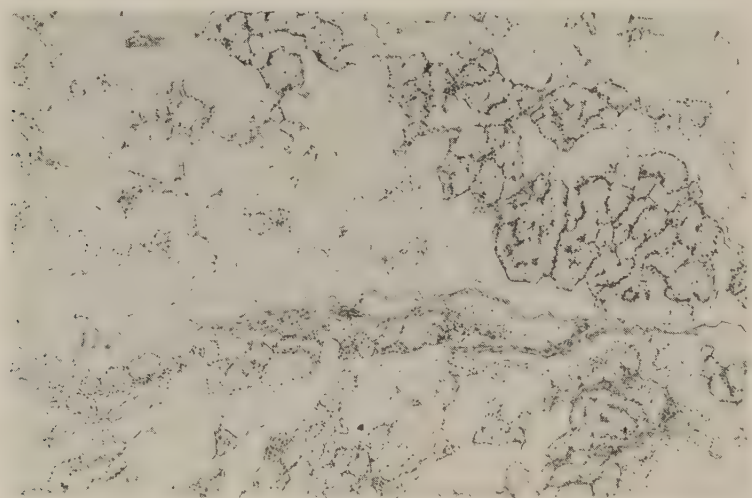


Fig 11. Hyperplasia (waterclear cell type) in a gland still showing areas with abundant stromal fat. There was, however, a total lack of intraparenchymal fat. Two other glands removed were quite solid (H&E, X40).

the adenoma diagnosis was correct with one exception which is not yet clarified to its nature. Even if this single case is a diagnostic error we consider the method highly reliable from a practical standpoint, and we think a "false normal" rate of 1 % is a quite acceptable cost for the benefits achieved by neck explorations limited to one side in adenoma cases (see below).

To be a useful routine method for limitation of parathyroid exploration the rate of equivocal findings in the diagnostically essential grossly normal glands also has to be reasonably low. Total elimination of this problem is not to be expected when working with biologic material, and we think the rate of 8 % in this series of primary hyperparathyroidism is quite acceptable. Development of fat staining techniques free from fat dissolving agents (5) may further reduce this figure.

As a practical consequence of our experience in this field we use since five years a special surgical strategy implying unilateral parathyroidectomy in cases with adenoma diagnosis. The application of this strategy and a thorough evaluation of its benefits compared to conventional surgery has been presented in detail previously (6,7). In short this strategy is applied as follows. If one enlarged gland and one grossly normal gland are found on the first side explored, both are removed for microscopic diagnosis including fat staining on frozen sections. If the small gland is normal, i.e. inactive, this implies that the enlarged gland is an adenoma and the operation is terminated. If not only the enlarged but also the presumed normal gland show signs of secretory activity this implies hyperplasia and the other side is explored. If two obviously abnormal glands are found on the first side a bilateral exploration is performed before any tissue is removed. If the two glands on the first side appear grossly normal they are left without biopsy and the other side is explored. If one enlarged and one grossly normal gland are found contralaterally they are both removed for microscopic diagnosis. In our material the gain in time of surgery and anesthesia is on an average 20 % when the operation is limited to one side, and there is also a significantly lower frequency and shorter duration of temporary postoperative hypocalcemia as well as total elimination of the risk for permanent hypoparathyroidism.

Besides the decreased risk for complications there are two things that make the possibility to limit parathyroid surgery in adenoma cases most interesting. Firstly, a solitary adenoma is by far the most common cause of primary hyperparathyroidism, and secondly, new technical achievements - especially high-resolution ultrasonography and high-resolution computerized tomography (15) - have shown to be very useful for preoperative localization of parathyroid adenomas. The first promising experiences of magnetic resonance in this context were recently published (16). An optimal utilization of these facilities in the management of parathyroid disease is not achieved by conventional surgery but demands new methods like those described here.

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*II. Unilateral parathyroidectomy in hyperparathyroidism
due to single adenoma*

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ABSTRACT

As a general principle in the treatment of primary hyperparathyroidism due to single adenoma, unilateral parathyroidectomy was applied to 50 patients and compared with another group of 50 conventionally explored patients. Twenty-five patients were explored only on the "adenoma" side. The other 25 patients were explored on both sides, avoiding biopsies at the first. In the conventionally explored patients, the adenoma was removed and one to three normal glands were biopsied. Oil-red-O technique was used in the intraoperative microscopical examination. The patients in whom the operation could be limited to the "adenoma" side had a statistically more favorable situation concerning early postoperative hypocalcemia, length of operation time, and need for calcium and vitamin D substitution. The principle of unilateral parathyroidectomy in conjunction with intraoperative oil-red-O staining technique is advocated in hyperparathyroidism due to single adenoma because it offers more reliable preoperative distinction between uni- and multi-glandular involvement, reduced operation time, decreased risk for complication, reduced early hypocalcemia, and more favorable technical conditions for reoperation.

INTRODUCTION

The aim of the surgical procedure in hyperparathyroidism due to single adenoma is to remove the tumor and as little as possible of the remaining normal glandular tissue. A major diagnostic dilemma during operation, is to decide whether the macroscopical tumor really represents a solitary lesion or if it is part of a multiglandular disease, in which case a more extensive surgical procedure is required. To cope with this dilemma, microscopic investigation of grossly normal parathyroid tissue is necessary.

Various surgical approaches to this problem have been advocated from removal of the adenomatous gland (22) to an obligatory 3.5 gland excision (17). In multiglandular disease, either with or without connection to MEN syndrome, most authors agree that at least a subtotal parathyroidectomy should be performed. However, if the choice of surgical procedure is based solely on the macroscopic appearance of the glands, unpleasant surprises may occur at the microscopic examination, indicating that the grossly normal-looking gland really shows microscopic signs of nodular hyperplasia.

In the case of a single adenoma, removal of this and bioptic identification of the other glands is the procedure used most frequently (2,10,12,19). The extirpation of the tumor-bearing gland, leaving two or three glands identified but intact, is preferred by others (8,9,22).

The introduction of a peroperative fat-staining method on frozen sections by Roth and Gallagher (21) in 1976 and later, the oil-red-O modification as described by Ljungberg et al (14), have offered improved possibilities to distinguish between single adenoma and multiglandular hyperplastic involvement of the parathyroid organ. This method adds a functional dimension to the morphologic examination because the accumulation of intracellular fat droplets indicates suppressed parathyroid function and reduction of fat droplets hyperactivity as seen in adenoma or hyperplasia.

As a consequence of this improved diagnostic procedure, a new surgical strategy implying unilateral parathyroidectomy has been applied in the treatment of primary hyperparathyroidism due to

single adenoma. The present investigation was undertaken to assess the clinical significance of the new surgical principle.

DIAGNOSTIC CRITERIA

Single adenoma means that the microscopic demonstration of one tumor containing chief cells shows lack of suppression with oil-red-O technique, i.e., intracytoplasmic fat droplets are absent or sparse. In the same gland, a rim of normal suppressed chief cells, i.e., chief cells filled with intracytoplasmic fat droplets, is present.

The diagnosis of multiple adenomata is characterized by the presence of adenoma as described above in more than one gland, and the concomitant demonstration of one whole macroscopically normal gland that microscopically shows chief cells filled with intracellular fat droplets as an indication of suppression.

Nodular hyperplasia is defined as a state in which multiple nodules of chief cells, devoid of intracellular fat droplets, occur intermingled with areas containing chief cells with an abundance of intracellular fat droplets as an indication of suppression. Macroscopically normal glands with a uniform picture of suppressed chief cells are not found.

Diffuse hyperplasia is characterized by chief-cells that uniformly are devoid of intracellular fat droplets. Macroscopically normal glands with signs of suppression are not found.

MATERIAL AND METHODS

Four groups, each consisting of 25 patients with primary hyperparathyroidism due to single adenoma were compared as to postoperative S-calcium level, postoperative need of substitution treatment, operation time, and late results. All patients had a single adenoma removed. None had previously undergone either thyroid or parathyroid surgery. Indication for surgical treatment was based on clinical characteristics and conventional diagnostic means, elevated S-calcium, S-PTH, and increased urinary output of cyclic AMP. The patients did not belong to families with hyperparathyroidism nor were any of them afflicted with multiple endocrine neoplasia. The mean age was 61.5 and did not differ significantly bet-

ween the various groups. Twenty-three were male (8, 4, 5, and 6 respectively of each group).

The patients were selected consecutively from the clinical material: the individuals of group I and II from the beginning of the period during which the unilateral parathyroidectomy was applied; the individuals of group III and IV from the time preceding and partly overlapping this period.

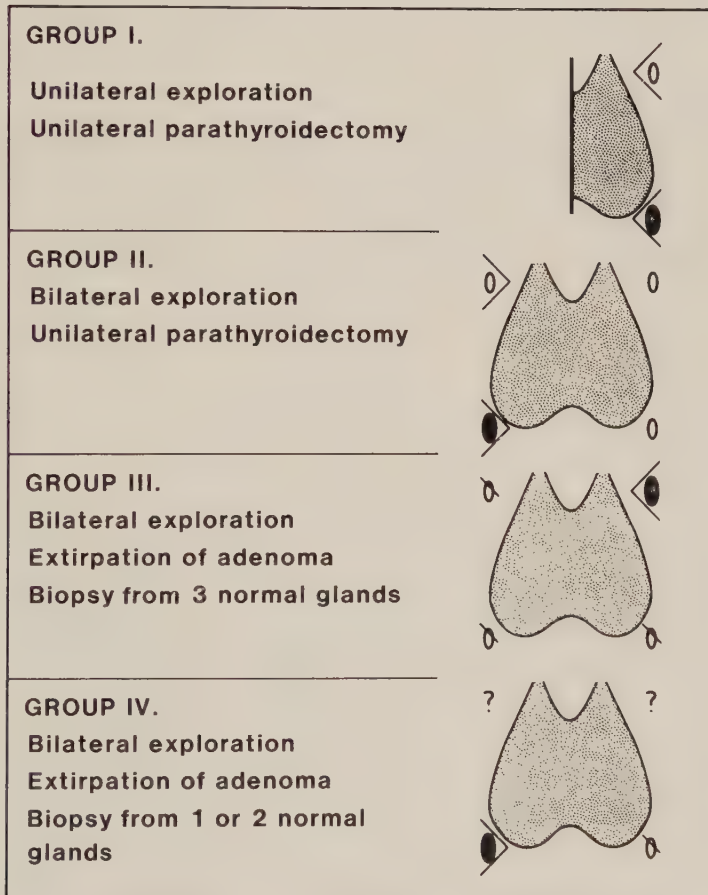


Fig 1. The four surgical procedures for hyperparathyroidism due to single adenoma that were studied.

In group I the exploration was limited to the first side to be explored. Both the adenoma and the normal gland of this side were removed. The contralateral side was not explored (Fig 1). Group II comprised patients in whom the side explored first revealed two grossly normal parathyroids. These were left intact. The adenoma and the fourth normal gland were found at the other side in which unilateral parathyroidectomy was performed. Group III comprised patients who were explored bilaterally and in whom extirpation of the adenoma and identification by incisional biopsy of three normal glands were performed. Group IV comprised patients in whom a parathyroid adenoma was extirpated and identification by incisional biopsy of all three normal glands was attempted, but was successful in only one or two glands.

Biopsy techniques

Excisional biopsy. The gland was dissected free, and the hilar vessels were isolated, divided, and ligated. Surrounding fat tissue was removed, and the normal gland was weighed immediately after removal. The weight of the removed normal glands varied between 18 and 62 mg. Following the weighing procedure, the gland was delivered in saline for pathologic examination.

Incisional biopsy. Part of the gland, usually one of the poles, was dissected free, and an incision of less than 1/4 of the gland was performed by use of eye-scissors. Immediately following the incision, the incised part of the gland was weighed. The weight of the specimen never exceeded 10 mg. Immediately following the weighing procedure, the specimen was delivered in saline to the pathology department for frozen section examination.

In groups I and II the removed specimens were investigated at operation with conventional hematoxylin-eosin method and the oil-red-O staining technique performed on frozen sections (14). In groups III and IV, the tissue was studied with hematoxylin-eosin only.

S-calcium determinations were performed four hours after operation and during the following postoperative days. The blood samples were withdrawn under fasting conditions in the morning before the patients had received any peroral or intravenous calcium treatment.

Peroral calcium treatment was given as fervescent calcium tablets of 0.5 g. The patients were informed of the early signs and symptoms of hypocalcemia and were instructed to take peroral calcium whenever subjective symptoms appeared. If hypocalcemia was prolonged for more than two weeks or calcium treatment was insufficient, vitamin D treatment was instituted.

The follow-up period was two to four years in groups I and II and four to seven years in groups III and IV. Wilcoxon's rank sum correlation test was used for the statistical analysis.

RESULTS

The postoperative S-calcium response is depicted in Figure 2. The mean preoperative calcium levels of the groups were 2.86, 2.95, 2.94, and 2.95 respectively (normal range 2.20-2.60 mmol/l). These mean values did not differ significantly. Hypercalcemia was eliminated in all groups during the first postoperative day.

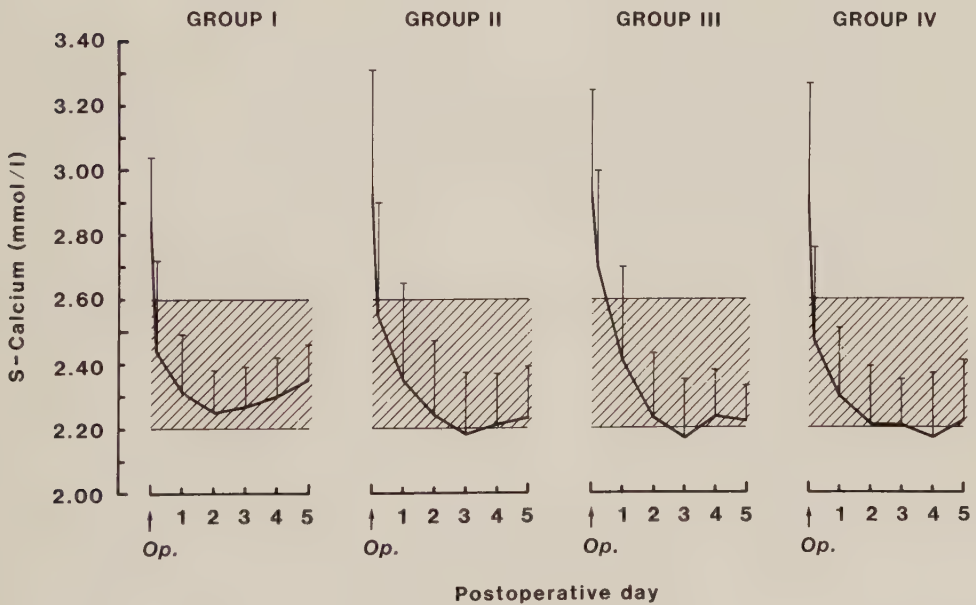


Fig 2. Early postoperative S-Calcium response to various surgical procedures for hyperparathyroidism due to single adenoma. Mean values $\pm$ SD.

The most marked depression of S-calcium was observed at the second postoperative day in group I, at the third day in groups II and III, and at the fourth day in group IV. The lowest mean value for group I was 2.24 and in the other groups 2.19, 2.17, and 2.18 respectively. At the fifth postoperative day, mean S-calcium was 2.34 in group I and 2.23, 2.22 and 2.23 respectively in the other groups.

The frequency of hypocalcemic days related to the degree of hypocalcemia in the four groups is depicted in Figure 3. In group I 19% of the postoperative days with available S-calcium determinations was hypocalcemic. The corresponding figures for the other groups were 34, 38, and 36% respectively. S-calcium values below 2.00 mmol/l were not recorded in group I. In the other groups such values were observed at seven, three, and three days respectively. Mean values for the hypocalcemic deviation are depicted graphically in Figure 4. The degree of hypocalcemia was the same in all treatment groups in the initial postoperative phase. At the fifth day, the hypocalcemia was significantly less in group I.

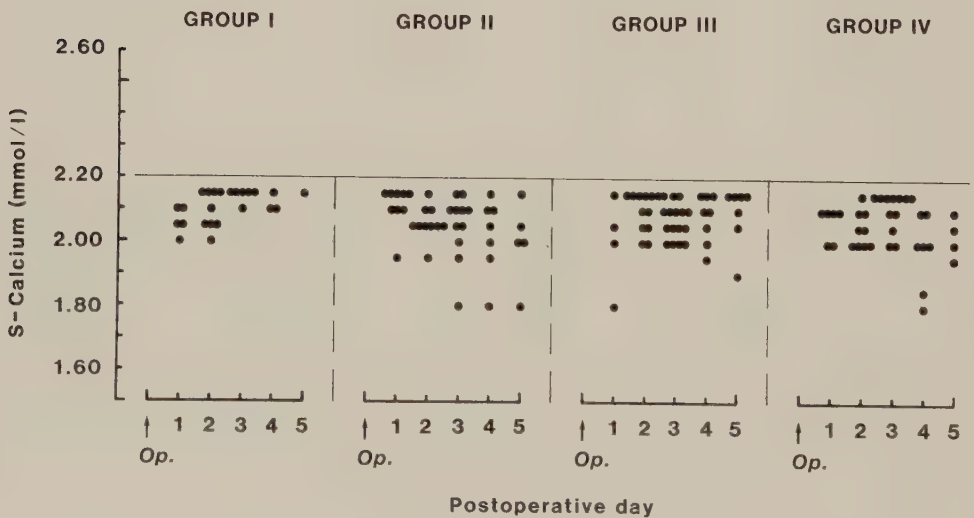


Fig 3. Individual occurrence and degree of early postoperative hypocalcemia following various surgical procedures for hyperparathyroidism due to single adenoma.

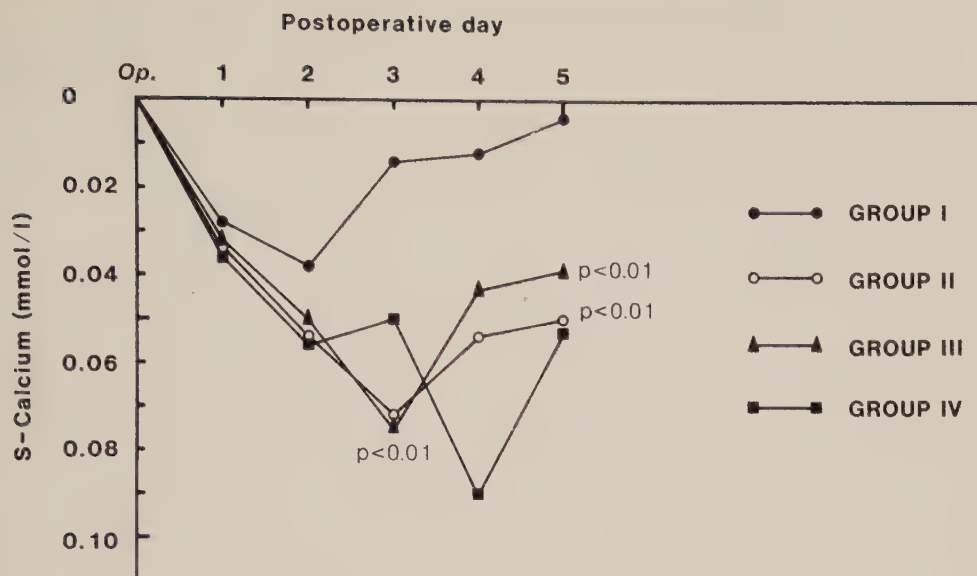


Fig 4. Mean hypocalcemic deviation in the early postoperative period following various surgical procedures for hyperparathyroidism due to single adenoma.

All patients were normocalcemic at the examination four weeks after the operation. Two to seven years follow-up is available in all patients. No case of recurring hyperparathyroidism has been reported. Neither has any instance of late hypocalcemia been observed. Vocal cord function was normal in all patients at the one-year follow-up.

Table 1. Postoperative Treatment for Hypocalcemia after Various Surgical Procedures for Hyperparathyroidism. Each Group Includes 25 Patients.

Surgical Procedure	Transient Peroral Calcium	Permanent Vitamin D
Group I	2	0
Group II	8	0
Group III	9	1
Group IV	9	1

Transient peroral calcium treatment was given to two of the patients in group I (Table 1). In the other groups, where both sides were explored, peroral calcium was given to eight, nine, and nine patients respectively. In addition, one patient in each group III and IV had a permanent need of vitamin D at the follow-up.

The length of the surgical procedure comprehended time from skin incision to the final stitch, including the frozen section examination (Table 2). Mean length was 93 minutes for the patients explored unilaterally as compared to 115 and 116 minutes for groups II and III. The incompletely explored patients in group IV had a mean length of operation time of 164 minutes. A statistical comparison between the groups showed significantly shorter operation time for group I. The postoperative care period had a mean length of 5.6 days. There were no significant differences between the groups.

Table 2. Length of Surgical Procedure (Min)

Surgical Procedure	Mean Operation Time
Group I	93
Group II	115
Group III	116
Group IV	164

DISCUSSION

Peroperative histopathologic examination is important for the identification of parathyroid tissue and the distinction between uni- and multiglandular involvement. Hematoxylin-eosin staining is a reliable method for the identification of parathyroid tissue. For the distinction between various types of parathyroid pathologic involvement it is sometimes inadequate. As this distinction is important for the choice of surgical procedure, the addition of the oil-red-O technique to the peroperative diagnosis represents a considerable improvement. In addition to the morphologic dimension of the examination, the oil-red-O technique adds a functional dimen-

sion. Occurrence of intracytoplasmic fat droplets indicates suppressed chief cells and absence of intracytoplasmic fat droplets signifies hyperfunction.

The rim of normal parathyroid tissue with suppressed chief cells found in an adenomatous gland is of great value for the diagnosis of adenoma. The characteristic picture of a mixture of hyperfunctioning nodules intermingled with areas of normal suppressed chief-cells is a strong diagnostic indication for nodular hyperplasia. In macroscopically normal glands, the finding of minute hyperfunctioning nodules suggests the presence of nodular hyperplasia. Particularly useful is the technique in the diagnosis of diffuse hyperplasia in macroscopically normal glands. The chief cells of these glands are devoid of intracellular fat droplets. In a previous report, Ljungberg et al (14) could demonstrate that of 39 cases with adenoma 38 showed the typical picture of adenoma chief cells devoid of fat droplets in combination with a rim of normal chief cells with an abundance of fat droplets.

Chief cell hyperplasia was first recognized as a cause of primary hyperparathyroidism by Cope et al (5) in 1958. They stressed the generalized involvement of all parathyroid glands as opposed to the adenoma disease. Still, in nodular hyperplasia only one gland may appear grossly abnormal whereas the remaining normal-looking glands at microscopic examination reveal minute hyperplastic foci indicating hyperfunction. So called "true recurrences" of hyperparathyroidism may represent such cases where only the grossly abnormal gland has been removed, whereas in the remaining glands areas of nodular hyperplasia have been allowed to develop into a clinical recurrence later on. The microscopic examination of one whole macroscopically normal gland should reduce the risk to oversee minimal nodular changes.

Since the introduction of the oil-red-O technique, 32 cases of primary hyperparathyroidism due to hyperplasia were diagnosed. They were all treated with subtotal parathyroidectomy. In 10 of these cases an enlarged gland was found together with a grossly normal gland at the first explored side, simulating single adenoma disease macroscopically. In these cases, however, the peroperative microscopic examination revealed involvement of the grossly normal

gland, indicating hyperplastic disease. Subsequent exploration of the other side showed additional abnormal glands.

When applying the surgical strategy of unilateral parathyroidectomy, it is crucial to exclude the possibility of a multi-glandular disease at operation. For that reason a one-sided parathyroid operation should not be performed with less than the microscopic identification of one whole normal gland.

In order to test the principles of unilateral parathyroidectomy against the conventional "four gland exploration" procedure, four groups of patients with hyperparathyroidism due to single adenoma were compared. In groups I and II the principle of unilateral parathyroidectomy was applied; in groups III and IV the principle of exploring all four glands was used.

Removal of the parathyroid adenoma resulted in a prompt elimination of the hypercalcemia in all groups. The drop of the postoperative S-calcium was more marked in groups II, III, and IV, suggesting that the approach in group I in which only one side had been explored was less traumatic. Also the number of postoperative hypocalcemic days were fewer, and the absolute level of hypocalcemia was less pronounced following the unilateral exploration. The increased need for peroral calcium medication or vitamin-D substitution in groups II, III, and IV also indicates that the damage is reduced by restricting the exploration to one side. Particularly notable is that the mere identification of the glands without biopsies as in group II resulted in a similar frequency of postoperative hypocalcemia as seen in group III, where incisional biopsies of all three normal glands were performed. This clearly demonstrates that even an atraumatic identification procedure carries a risk of at least a transient hypofunction of the healthy glands. The lack of difference in the frequency of hypocalcemia between groups II and III supports the opinion that the identification procedure is equally traumatic as the biopsies. Removal of one whole normal gland did not seem to influence negatively the postoperative S-calcium level. The chief cell mass in one normal gland should be approximately the same as biopsies from three glands.

In the present material the frozen sections were studied both with conventional hematoxylin-eosin and the intracellular fat staining technique. In spite of the somewhat increased time consumption for the mere staining procedure the operation time for the unilateral exploration was less than in the other groups. The fourth group in which the exploration was incomplete because of lack of identification of one or two normal glands the operation time was significantly prolonged.

In 12 of the 25 patients in group I, no hypocalcemic postoperative days occurred. This suggests that the normal glands of these patients retained a sufficient functionary capacity to avoid hypocalcemia already during the first postoperative days.

The variation in intracellular fat droplet content recently reported (14) possibly reflects a varying degree of suppressed chief cell function. The correlation between grade of hypocalcemia after operation and degree of suppression as reflected in the appearance of intracytoplasmatic fat droplets is presently studied. Dekker et al (8) recently reported on the therapeutic impact of the oil-red-O, and they concluded that the surgically more conservative approach is justified in patients with primary hyperparathyroidism.

Roth, Wang, and Potts (20) have previously described a unilateral approach in hyperparathyroidism. If one abnormal and one normal gland is found on the first side to be explored, they advocate the removal of the adenoma and a 0.1-0.2 cm biopsy of the normal gland. In our opinion, the removal of one whole gland facilitates the evaluation of hyperplasia for the pathologist, particularly if the oil-red-O staining technique is used as it allows a functional evaluation of the normal chief cells. We agree with these authors that the exploration should continue on the contralateral side if any signs of multiglandular involvement are revealed at the histopathologic examination of the glands from the first explored side. We also agree that the exploration should be extended to the contralateral side if one or two normal glands are found on the first side. In this situation we do not biopsy the macroscopically normal-looking glands of the first explored side if the adenoma and a macroscopically normal gland are found at the contralateral side. If only one adenomatous gland is found, one whole gland from the

contralateral side is removed provided there are two identified. We thus try to leave the glands of one side without biopsies and remove all parathyroid tissue at the "adenoma side".

Postoperative hypocalcemia, even of mild degree, is a miserable state not only because of tetany but also as a result of the anxiety that accompanies it. The rapidly increasing extent of parathyroid surgical activity necessitates the formulation of simple and safe surgical methods. A more conservative approach to the parathyroid neck exploration has recently been advocated by Edis et al (9). They compared neck explorations in which biopsies were used extensively with operations in which the enlarged gland was removed with or without biopsy of one normal-sized gland.

Symptomatic hypocalcemia requiring treatment occurred in 24% of the more extensively explored patients as compared to 4% in the more conservatively treated group. The more extensive explorations did not yield any higher cure rate.

In a Scandinavian survey of parathyroid surgical activity during 1975, 652 patients, operated upon in various hospitals around Scandinavia, were studied. The best results were obtained when only the adenoma was removed. In this group 9% were hypocalcemic at the discharge from the hospital. If biopsy was done on one or more glands, the corresponding figure was doubled (11). These figures indicate that the principle of identifying and biopsying all glands should be critically reconsidered.

Unilateral parathyroidectomy should be reserved for patients with primary hyperparathyroidism due to a single adenoma who previously have not undergone a neck exploration. In re-explorations either after previous parathyroid surgery or thyroid surgery, the exploration should be adjusted to the individual situation. In these patients, removal of one whole normal gland might be hazardous as healthy parathyroid tissue might have been removed with or without intention at previous operations. Also, in patients with familial hyperparathyroidism as well as in patients with multiple endocrine, adenopathy unilateral parathyroidectomy should not be used. If these different types of hyperparathyroidism are excluded, the majority of cases still remains, i.e., patients with hyperpara-

thyroidism due to a single adenoma. In the clinical material of our institution from the latest six years, it would have been possible to employ the surgical principle of unilateral parathyroidectomy in 70% of all cases with primary hyperparathyroidism.

Since the introduction of the oil-red-O method in 1976, 186 patients undergoing various forms of parathyroid surgery have been investigated. Multiple adenomata fulfilling the criteria given above have not been found in any patient. In the 50 patients included in the present study, no case of hypercalcemia could be demonstrated at the follow-up two to four years after the operation.

Multiple parathyroid adenomata have been reported in several series of patients with primary hyperparathyroidism. A review of eight patient-materials after 1958 gives a frequency of 1.9% - 5% (1-4,7,15,18,23). Harness et al (13) reports a 1.7% incidence of double adenomas. In a recent report on 199 patients with primary hyperparathyroidism, Block et al (3) noted seven patients with enlargement of only two parathyroid glands. In the first 200 patients treated surgically for hyperparathyroidism at the Massachusetts General Hospital, 5% of double adenomas were found. Wang and Rieder (23) reported in 1978 that of 13 of the patients with double adenomas found at the Massachusetts General Hospital through 1958, six eventually proved to be primary chief cell hyperplasia, and the diagnosis has not been conclusively substantiated in the other seven. Since 1958, they were not able to find a single case of double adenomata. Cope (6) has stated that "it is such a biological feat to make one hyperfunctioning adenoma that a coincidental second hypersecreting neoplasm is highly unlikely." None of these reports have used the strict diagnostic criteria of the present study. The demonstration of one whole, macroscopically normal gland with a uniform picture of suppressed chief-cells as indicated by abundance of fat droplets must be considered a necessary prerequisite for the diagnosis of multiple adenomata. Moreover, the glands in which the adenomata are found must exhibit a rim of normal suppressed chief cells.

Even if no case could be found in the present material, multiple adenomata might exist, although in a frequency probably less

than 1%. As the chance of having two adenomata at the same side is as big as finding one at each side, the risk of missing a second adenoma by applying unilateral parathyroidectomy is reduced by 50%. Further, should it occur, the reoperation can be performed in previously unexplored tissue.

The elimination of hypercalcemia and the absence of recurrence during the follow-up after unilateral parathyroidectomy indicates that this method can be used safely with regard to curability. Moreover, even if the differences were small between the groups explored according to different surgical strategies the postoperative hypocalcemia was less pronounced in the patients in whom only one side had been explored.

In conclusion, the data of the present report suggest that unilateral parathyroidectomy as a surgical principle based upon preoperative functional and morphologic evaluation by oil-red-O technique is a good choice in the treatment of hyperparathyroidism due to single adenoma. It offers:

1. More reliable peroperative distinction between uni- and multi-glandular involvement.
2. Reduced hypocalcemia in the early postoperative course, both as to frequency and degree.
3. Decreased risk for complications in terms of nerve damage and persisting hypoparathyroidism.
4. Reduced operation time.
5. More favorable technical conditions if a reoperation should be necessary.

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Errata: Errors made at printing of this paper in Annals of Surgery have here been corrected.

*III. Surgical strategy in hyperparathyroidism
due to solitary adenoma*

S. Tibblin, A. Bondeson, L. Bondeson, O. Ljungberg

ABSTRACT

Based on the postulate that parathyroid adenoma is practically always a solitary lesion, unilateral parathyroidectomy including the homolateral normal parathyroid was applied as a principle in the treatment of this form of primary hyperparathyroidism. The exploration was confined to the adenoma side if this was the first to be explored. Intraoperative oil-red-O staining of frozen sections was used to exclude the possibility of a multiglandular involvement. This principle was applied in a consecutive series of 102 patients operated for hyperparathyroidism from 1977 to 1981 and diagnosed as parathyroid adenoma. In 43 patients where the abnormal gland was found on the side explored first, unilateral parathyroidectomy was performed on that side, avoiding exploration of the contralateral side. In 45 patients where normal glands were found on the side first explored, unilateral parathyroidectomy was performed on the contralateral side. In 14 patients other types of operations were performed as the above-mentioned principle could not be achieved. At follow-up 1 to 5 years after surgery, no cases of hypercalcemia were recorded. The results of the different operations were compared as to early and late hypocalcemia. Early hypocalcemia was more pronounced after a bilateral exploration. Two of the patients who had an atypical operation had a permanent need for vitamin D in order to maintain an adequate serum calcium level. Surgical principles for various possible exploratory findings are outlined. These are based upon the idea of performing a unilateral parathyroidectomy whenever intraoperative oil-red-O staining excludes multiglandular involvement as a cause for the hyperparathyroidism.

INTRODUCTION

Solitary parathyroid adenoma is the most frequent cause of primary hyperparathyroidism. In the choice of surgical procedures, the surgeon has to cruise between the Scylla of hypocalcemia and the Charybdis of remaining hypercalcemia after surgery. In a previous report (1) we described a histopathologic method facilitating the distinction between uni- and multiglandular involvement by utilizing oil-red-O. Normal chief cells show an abundance of intracellular fat droplets, probably as an expression of inactivity. In the autonomously hyperfunctioning tissue, on the contrary, such intracellular fat droplets are markedly reduced in amount or absent. Partly as a consequence of this improved histopathologic methodology, we have modified our surgical technique in patients with hyperparathyroidism due to a solitary adenoma. Instead of striving to explore and biopsy all four glands, we limit the operation, if possible, to the adenoma side in which all parathyroid tissue is removed. In another report (2) we have compared the results of this new technique with the more conventional four gland exploration. In the current report a critical analysis of the clinical application of this new surgical measure is presented. The aim of this investigation was to report the applicability of the new surgical strategy in a routine clinical practice and the results as to postoperative morbidity. Based upon these experiences we also propose some outlines for the surgical exploration and treatment of hyperparathyroidism due to solitary adenoma.

MATERIAL

A consecutive series of 102 patients were treated surgically for primary hyperparathyroidism due to solitary adenoma. None of the patients had previously been explored for hyperparathyroidism. The first patients of the series were operated on in 1977 and the last in December 1981. During this period the idea of making a unilateral parathyroidectomy gradually became the method of choice in the surgical treatment of parathyroid adenoma. All patients in whom this surgical principle was aimed at (72%) are included in the series. The mean age was 63 years. Seventy-five per cent were women. Mean preoperative serum calcium (S-calcium) was 2.92. All patients were hypercalcemic before surgery (normal range 2.20-2.60 mmol/l). The oldest patient was 88 and the youngest 17 years old.

The presenting symptom was renal in 24 patients. Symptoms from the central nervous system were the presenting complaints in 44 patients; in 11 patients gastrointestinal symptoms were dominating; and 15 patients were considered asymptomatic at the preoperative evaluation. In eight patients cardiovascular symptoms, mainly due to arterial hypertension, were the main presenting symptoms. For comparison a control of 58 patients with similar age and sex distribution operated for hyperparathyroidism due to solitary adenoma was used. These patients were operated with bioptic identification of three normal glands and removal of the adenoma.

METHODS

The principle for the surgical method was to remove the adenomatous gland and a whole macroscopically normal gland. If possible, this was performed as a unilateral parathyroidectomy, thus clearing the adenoma side from all parathyroid tissue. If the adenoma was not found on the first side to be explored, the contralateral side of the neck was explored. If the adenoma and the macroscopically normal whole gland were found on the contralateral side a unilateral parathyroidectomy was performed on that side, thus leaving the two macroscopically normal glands of the first side unbiopsied. If, in addition to the adenoma, only one normal gland was found heterolaterally, an incisional biopsy of this was performed. A "typical operation" was defined as a neck exploration in which either the adenoma was found on the first side to be explored and in which a unilateral parathyroidectomy of this side was performed without exploring the other side, or two macroscopically normal glands were found on the first side to be explored and a unilateral parathyroidectomy was done on the contralateral side. "Atypical operations" were defined as explorations in which other types of surgical procedures were performed.

For the intraoperative histopathologic examination the oil-red-O method was invariably used on all parathyroid tissue specimens removed. The details of the staining procedure have been described elsewhere (1). Great care was taken to localize and indicate the remnant of the normal parathyroid tissue in the adenomatous gland before the specimen was delivered to the pathologist. To facilitate the identification of this part of the gland, dissection of the

parathyroid hilar vessels was performed and, when found, marked by a ligature.

S-calcium was followed daily during five days. After surgery the blood samples for S-calcium determination were taken under fasting conditions.

Before the operation the patient had been informed about early signs and symptoms of hypocalcemia that may occur after surgery and had been instructed to take oral effervescent calcium tablets when such subjective symptoms occurred.

Following the surgical procedure the patients were reexamined as to general condition and S-calcium one month and one year after surgery. During the first postoperative month all patients underwent a vocal cord examination.

Sixty-one of the surgical procedures were performed by a senior endocrine surgeon. The other 41 operations were performed by three general surgeons undergoing training in parathyroid surgery. Their experience of parathyroid surgery ranged from one to three years.

For statistical analysis of the results the rank sum correlation test according to Mann-Whitney was used.

Table I. Surgical Procedures

Surgical Procedure	No. of Patients
Unilateral parathyroidectomy + unilateral exploration	43
Unilateral parathyroidectomy + bilateral exploration	45
Atypical procedures	14

RESULTS

The application of the above-mentioned surgical principles resulted in a unilateral exploration and unilateral parathyroidectomy in 43 patients (table I). In 45 patients two macroscopically normal glands were identified at the first side to be explored resulting in an exploration of the contralateral side in which an adenoma and a normal parathyroid gland were found and removed. The remaining 14 patients had varying types of atypical operations. In four of them only one normal gland was found in addition to the adenoma at exploration of both sides. In two of these an incisional biopsy of the macroscopically normal gland was performed and the demonstration of suppressed normal chief cells was made. In the other two, the whole macroscopically normal gland was removed. In the other eight patients at least two glands in addition to the adenoma could be localized. In seven of these at least one macroscopically normal gland was left *in situ* without biopsy. In the eighth patient one gland was not even explored. In this patient two glands had been removed at the first side, one of which had been suspected for an adenoma. The microscopic examination, however, revealed two normal glands with signs of suppression as judged from the oil-red-O stain. At the exploration of the other side the adenoma was encountered in the lower gland, which was the first position to be explored on this side. As two normal, suppressed glands had been removed at the first side, the upper right was not looked for. In two patients the homolateral macroscopically normal gland showed to be something else than parathyroid tissue. In both these patients a remnant of normal parathyroid tissue containing chief cells with increased amount of fat droplets could be demonstrated in the adenomatous gland. These patients were not explored contralaterally. In one patient three glands were found at the first side to be explored. One of these was the adenomatous one. One of the two other normal-looking glands was removed; it was histologically normal with increased amount of parenchymal fat. The third gland on this side was never biopsied. It had a gross appearance of a normal parathyroid gland. The other side was also explored and two macroscopically normal-looking glands were found in classic positions. In two patients only one normal gland was found at the first side explored. On the other side the adenomatous gland and a normal gland were revealed. In both of these patients a unilateral parathyroidectomy was performed.

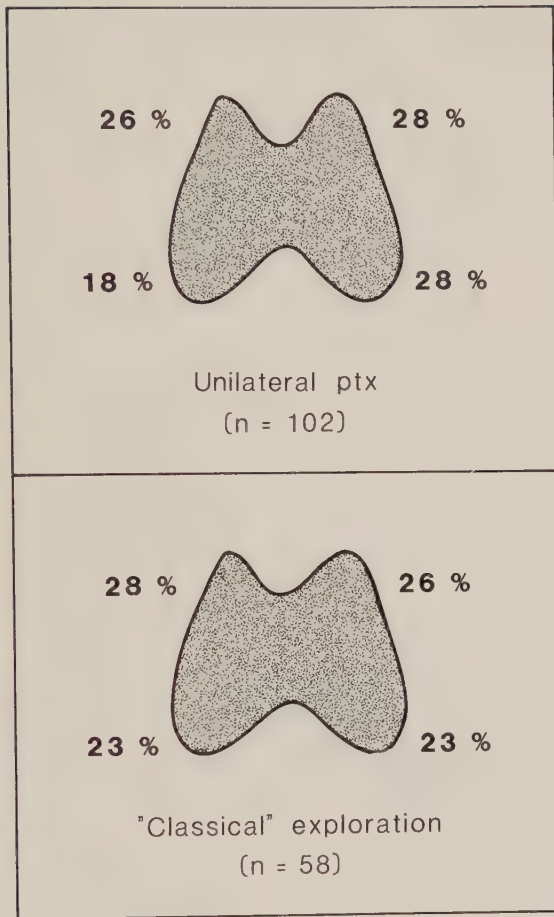


Fig 1. Distribution of the adenoma (%) at various positions in two patient materials operated for hyperparathyroidism due to solitary adenoma according to different surgical principles.

The position of the adenomas is depicted in figure 1. There was no preferential position. The localization of the parathyroid adenomas did not deviate significantly from the distribution of adenomas in the series of patients in which all four positions had been

explored. The "typical exploration" was not achieved in 14 patients. In figure 2, the frequency of unsuccessful explorations related to the four classic sites of the glands is depicted. The intended exploration was not successful in 5% including all four sites. Supernumerary glands were encountered in seven patients (7%) (fig 3). The distribution of these glands was the same for both sides. No patients had more than one supernumerary gland. Supernumerary glands were found more frequently in the series of explorations performed according to the new surgical strategy than in the series of classical explorations in which supernumerary glands were revealed in two per cent of the cases.

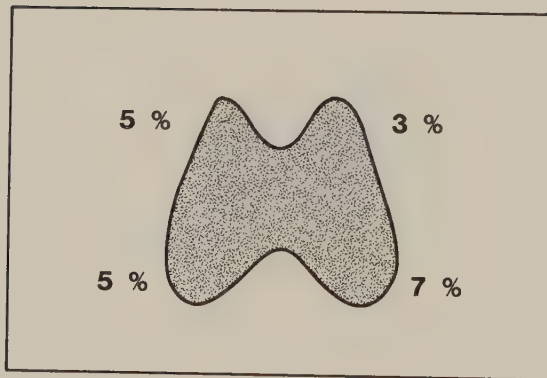


Fig 2. Frequency of unsuccessful explorations, related to the four classic parathyroid positions in 102 patients operated for hyperparathyroidism due to single adenoma.

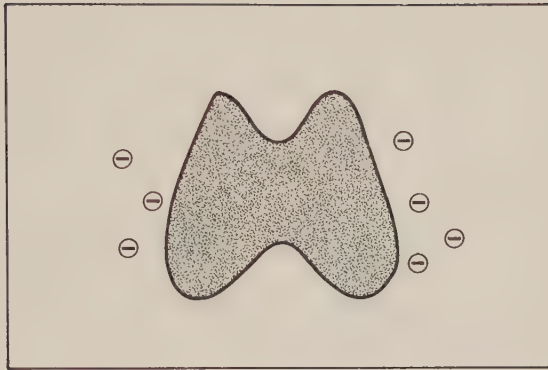


Fig 3. Supernumerary parathyroid glands were found in 7 of 102 patients operated for hyperparathyroidism due to solitary adenoma.

One whole macroscopically normal gland was obtained for histopathologic examination in 95 patients. The exploration was limited to one side in 43 patients. A "clean" side, i.e. removal of at least two homolateral glands, was achieved in 91 patients.

Early postoperative S-calcium changes

Figure 4 shows the postoperative S-calcium values for the two patient groups undergoing typical surgical procedure according to the outlined strategy. Forty-three patients with unilateral parathyroidectomy had 41 hypocalcemic days following a unilateral exploration corresponding to 19% of all postoperative days studied. In 45 patients exposed to a bilateral exploration and unilateral parathyroidectomy 24% of all postoperative days were hypocalcemic. In the patients explored bilaterally hypocalcemia was more pronounced during the third ($p < 0.01$) and the fourth day ($p < 0.05$).

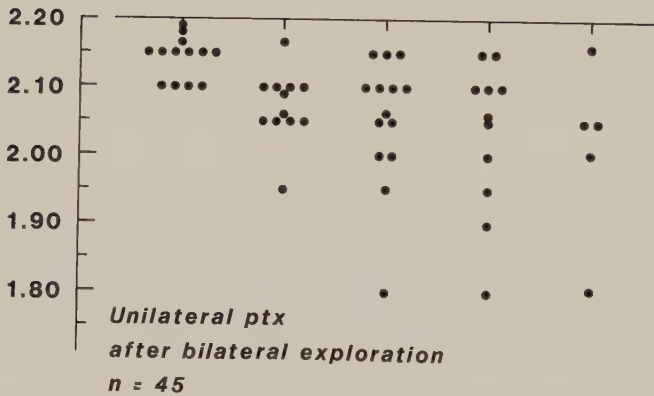
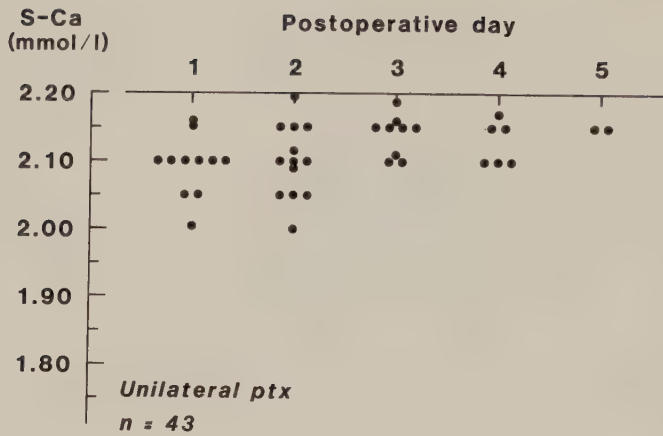


Fig 4. Postoperative hypocalcemic days (S-Ca; normal range 2.20 - 2.60 mmol/l) following unilateral parathyroidectomy without and with bilateral exploration. Each dot represents the hypocalcemic value of an individual patient.

The distribution of the patients according to the lowest S-calcium values recorded after surgery and related to the type of operation is given in figure 5. A hypocalcemic level was reached more often following neck explorations in which an atypical operation had been

performed. Postoperative S-calcium value below 2.00 mmol/l was not recorded in patients with a one-sided exploration.

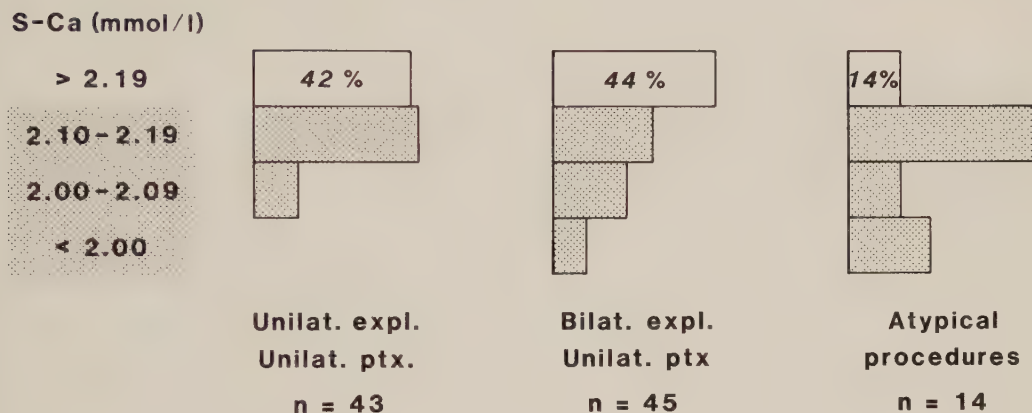


Fig 5. Distribution of the patients (%) according to the lowest serum calcium values recorded after surgery and related to the three different types of surgical procedures.

Mean levels of preoperative S-calcium were identical in the unilaterally and the bilaterally explored groups, and there was no significant difference when comparing patients with regard to presence or absence of early postoperative hypocalcemia (2.89 and 2.94 mmol/l, respectively). Mean levels of preoperative serum alkaline phosphatase (normal range 1.3 - 5.0 μ kat/l) were almost the same in the unilaterally and the bilaterally explored groups (4.2 and 4.4 μ kat/l, respectively) and there was no significant difference when comparing patients with regard to presence or absence of postoperative hypocalcemia (4.4 and 4.2 μ kat/l, respectively).

Occasional intake of calcium by mouth during the immediate postoperative period occurred frequently in the group treated with atypical procedures (5 of 14 patients). In the group bilaterally

explored but unilaterally parathyroidectomized, three patients required transient calcium treatment. This was needed by one patient only in the unilaterally explored group. A permanent or transient need of vitamin D did not occur in the patients operated with typical procedures regardless whether they had been explored uni- or bilaterally. In the series of 14 cases operated with atypical procedures, two required permanent vitamin D administration.

Late disturbances in S-calcium level

All patients were followed-up for at least one year. Late hypercalcemia was not recorded in any of the 102 patients. Two patients were taking vitamin D daily in order to maintain normocalcemia. In figures 6 to 8 the S-calcium levels before and one year after operation are given for each patient. In each of the three groups one patient shows a value at the upper limit of normal range one year after operation. These patients have now been followed for 3 to 4 years and they are all normocalcemic (2.50, 2.45, and 2.53 mmol/l, respectively).

Complications

No postoperative deaths occurred. Transient one-sided vocal cord palsy occurred in one patient of the uni- and bilaterally explored groups, respectively. A transient recurrent nerve palsy occurred in one of the 14 patients operated according to the atypical procedure.

Postoperative hemorrhage requiring a revision of the wound occurred in three patients unilaterally explored, in two patients bilaterally explored and operated with unilateral parathyroidectomy and in one patient belonging to the group of patients that was operated with other procedures.

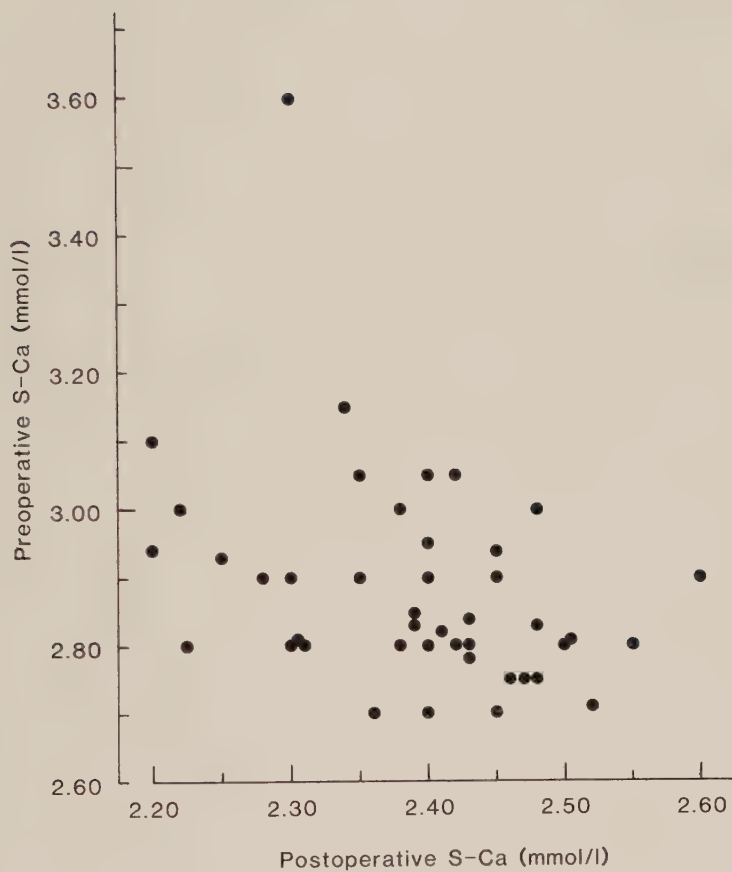


Fig 6. Preoperative serum calcium levels plotted against serum calcium levels one year after operation in 43 patients treated with unilateral neck exploration and parathyroidectomy.

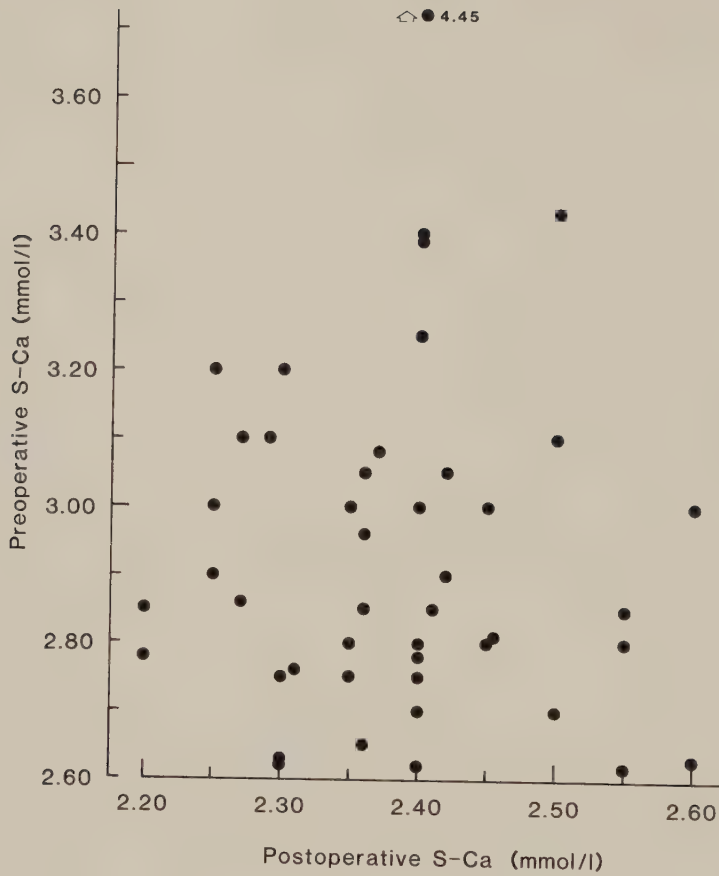


Fig 7. Preoperative serum calcium levels plotted against serum calcium levels one year after operation in 45 patients treated with unilateral parathyroidectomy after bilateral neck exploration.

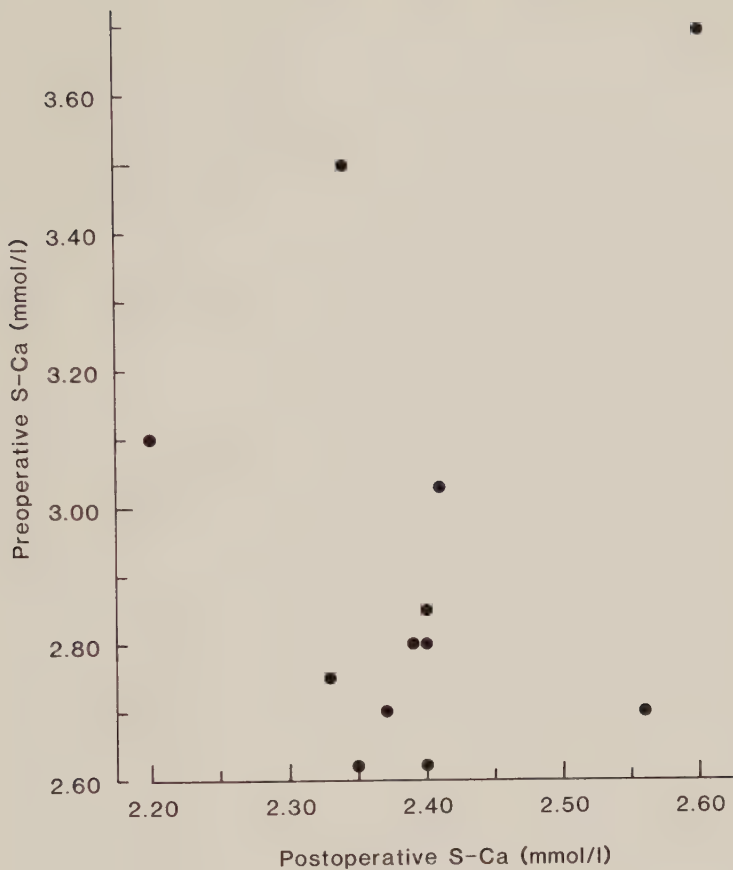


Fig 8. Preoperative serum calcium levels plotted against serum calcium levels one year after operation in 12 patients being without supplementary therapy after atypical parathyroidectomy.

Additional surgical procedures

In 22 patients a surgical procedure directed towards the thyroid gland was also performed. In the unilaterally explored group four patients were hemithyroidectomized and four were treated with smaller lobe resections. One-half of these patients showed transient postoperative hypocalcemia (altogether 6 hypocalcemic days). In the group treated with unilateral parathyroidectomy after bilateral exploration, two patients had bilateral subtotal thyroid-

ectomies, three were hemithyroidectomized, and four were treated with smaller lobe resections. All but one of these patients showed transient postoperative hypocalcemia (altogether 23 hypocalcemic days). In the atypically treated group, one total, one subtotal, and three unilateral thyroidectomies were performed. All but one of these patients developed postoperative hypocalcemia (altogether 13 hypocalcemic days except the case of total thyroidectomy, in which permanent hypocalcemia developed). It appears that thyroid surgery, especially in bilaterally explored patients, was accompanied with an increased risk for development of postoperative hypocalcemia.

Outlines for the surgical strategy in hyperparathyroidism due to solitary adenoma

Based upon the experience from 102 surgically treated cases of hyperparathyroidism due to solitary adenoma, the outlines given in table II are proposed. The exploration of the neck is performed stepwise completing the exploration of one side before proceeding to the other side. The exploration of one side is restricted to the locations in which parathyroid glands are usually found. If the lower gland can not be identified along the thyreothymic ligament, the upper part of the thymic remnant is explored. If the lower gland is not localized below the level of the inferior thyroid artery, the sheath of the common carotid artery is explored up to the level of the bifurcation. If the lower parathyroid gland remains unidentified, further localization attempts are abandoned at this stage. Mediastinal exploration or hemithyroidectomy is not indicated to find a non-identified normal parathyroid gland.

If the upper gland is not found the superior thyroid arterial branches are divided and ligated so the posterior aspect of the upper pole of the thyroid lobe can be thoroughly explored. If the upper parathyroid is the site of an adenoma it is frequently dislocated posteriorly, inferiorly along the esophageal wall pulling the arterial supply with it in the posterior mediastinum.

Table II. Outlines for the Surgical Strategy in Hyperparathyroidism due to Solitary Adenoma

Exploratory finding		Surgical procedure
First side	Second side	
$A_1 + N_1$	No expl.	$A_{1X} + N_{1X}$
A_1	$N_2 + N_2$ N_2 No glands	$A_{1X} + N_{2X}$ $A_{1X} + N_{2B}$ A_{1X}
$N_1 + N_1$	$A_2 + N_2$ A_2	$A_{2X} + N_{2X}$ $A_{2X} + N_{1X}$
N_1	$A_2 + N_2$ A_2	$A_{2X} + N_{2X}$ $A_{2X} + N_{1B}$
No glands	$A_2 + N_2$ A_2	$A_{2X} + N_{2B}$ A_{2X}
A Adenoma N Normal gland 1 First explored side		X Extirpation B Incisional biopsy 2 Second explored side

Ideally, the contralateral side should not be explored until two normal parathyroids have been identified at the first side. If the exploration of the first side reveals a solitary adenoma and a macroscopically normal gland, the parathyroid tissue of this side is excised and exploration of the contralateral side is avoided. In

all other findings of the first-side explorations, the contralateral side will be explored. The general rules for exploration of the opposite side are the same as mentioned for the first side, and hemithyroidectomy or mediastinal exploration through sternal split is not performed at the primary exploration.

If the exploration of the first side has revealed only an adenoma and no normal-looking parathyroid gland the contralateral side has to be explored. If two normal glands are found in this side, one is excised. If only one normal gland is found at the opposite side an incisional biopsy is taken from this gland.

If two normal parathyroid glands have been identified at the first side to be explored, the neck exploration has to be extended to the contralateral side. If an adenoma and a normal gland are found at this side, a unilateral parathyroidectomy is performed, thus clearing this side completely. The other possibility is that only the adenomatous gland is localized. The adenoma-bearing gland is then excised in conjunction with one macroscopically normal gland from the first side..

If the exploration of the first side reveals only one macroscopically normal gland the other side has to be explored. The finding of an adenoma-bearing gland and a macroscopically normal-looking gland results in a unilateral parathyroidectomy of the contralateral side. If, on the other hand, only an adenoma-bearing gland is found on the contra-lateral side the adenoma in conjunction with an incisional biopsy of the normal gland of the first side to be explored is removed.

The fifth possibility occurs when no glands are found at all at the first side to be explored. The exploration is now extended to the opposite side in which the ideal finding is an adenoma-bearing gland and a macroscopically normal-looking gland. In this situation the adenoma-bearing gland is removed and an incisional biopsy is performed at the homolaterally normal-looking gland. If only an adenoma-bearing gland is localized on the contralateral side, this is completely removed without further exploration.

A macroscopically normal-looking gland should not be removed or

biopsied until the adenoma-bearing gland has been found. If more than one normal-looking gland has been localized in addition to the adenoma-bearing gland, it is preferable to remove one whole such gland, if possible from the adenoma side. If only one macroscopically normal-looking gland is found, an incisional biopsy of this gland should be done.

DISCUSSION

The permanent relief of hypercalcemia with a normocalcemia maintained without medication is the goal for all surgical therapy of hyperparathyroidism. To achieve this ideal state, autonomous hyperfunctioning parathyroid tissue must be removed and normal, well-functioning parathyroid tissue must be left *in situ* to guarantee a proper preservation of the S-calcium level. In approximately 85 % of all cases, primary hyperparathyroidism is caused by a solitary adenoma. In the remaining 15 % the condition is caused by a multiglandular parathyroid disease. The degree of involvement of the four glands in this condition may vary considerably between different glands in the same patient. The non-enlarged parathyroid glands, however, exhibit a histopathologic picture that is identical with the one present in the enlarged glands. It is of great importance to decide during surgery the type and extent of the parathyroid disease in order to choose the adequate surgical procedure. If a solitary adenoma is present, theoretically, only the removal of this adenoma is required. If, on the other hand, a systemic involvement of all parathyroid glands is present, all enlarged glands, possibly even some of the macroscopically normal one(s), have to be removed.

The introduction of the oil-red-O staining method during surgery has contributed considerably to facilitate the distinction between solitary adenoma and multiglandular, systemic disease. As a consequence of this improvement, outlines of a new surgical strategy in hyperparathyroidism due to solitary adenoma are presented. The removal of the homolateral, macroscopically normal gland in conjunction with the adenoma-bearing gland - unilateral parathyroidectomy - offers definite advantages compared to other surgical approaches. The removal of one whole macroscopically normal gland gives the pathologist a better possibility to distinguish between adenoma and hyperplasia, particularly if the oil-red-O

staining technique is applied. Since hyperplasia may be extremely asymmetrical and affect only a minor part of a macroscopically ordinary gland, the representativity of a biopsy could always be questioned. Considering the sometimes very uneven distribution of stroma and parenchyma in normal glands, the fat-staining is of great help to determine whether a compact area in a normal-sized gland represents a hyperplastic focus or not. From a surgical point of view the clearing of one side is advantageous, should it ever be necessary to re-explore the parathyroid glands of the patient. If it had been possible to accurately localize the parathyroid adenoma before surgery the unilateral parathyroidectomy had always been possible to perform without exploring the other side.

Presently, ultrasound technique and simplified methods for phlebographic procedures are tested in order to make such a side-localization possible. During the period in which these surgical procedures were performed, however, localization studies were not available, making the chance to hit the adenoma side 50%. This is also reflected by the figures encountered in the clinical material. The site of the adenoma was distributed percentwise without any statistically significant difference in the four ordinary positions. In this consecutive series of 102 patients, mediastinal or otherwise considerably atypical location did not occur. The typical operation, i.e. unilateral parathyroidectomy with or without exploration of the other side with identification of two normal glands, was possible to perform in 88 of the 102 patients. In the remaining patients, various other exploration types were used. In two of these the contralateral side was never explored. In these two patients the macroscopical finding of a homolateral, normal parathyroid gland showed to be false by the intraoperative microscopical examination. Since the wound already had been closed and the anesthesia finished, the further surgical procedures were abandoned, particularly as the adenoma-bearing gland showed a normal parathyroid rest with chief cells filled with intracytoplasmic fat droplets as an indication of suppression. When we have identified and removed a gland interpreted as grossly normal, the operation wound is usually closed but anesthesia is continued until the intraoperative pathologic report has arrived. An analysis of the unsuccessfully explored positions showed that about five per cent had not been rightly localized. Supernumerary glands were

encountered in seven patients. This finding is in accordance with the detailed anatomic dissection studies in cadavers performed by Gilmour (3). The increased number of supernumerary glands found in the current study is a reflexion of a more meticulous dissection technique applied in the operations performed according to the new surgical tactics. In conventionally explored patients supernumerary glands occurred only in two per cent.

Early postoperative hypocalcemia was less pronounced in the patients explored unilaterally. This was true in the comparison both with patients bilaterally explored with typical unilateral parathyroidectomy and with patients operated bilaterally and with other surgical procedures. Even if early postoperative hypocalcemia usually later disappear, the more pronounced hypocalcemia is both quantitatively and qualitatively an indication of the degree of trauma rendered to the normal parathyroid tissue. In the current material only two patients became permanently hypocalcemic. Both of these had been operated with atypical procedures. With the unilateral exploration the risk of remaining hypocalcemia is virtually absent because there will always be two normal parathyroids on the opposite side. The knowledge of this fact is of importance in the evaluation of the postoperative course. The number of postoperative days can be reduced. Also, the risk of other complications that can occur in connection with neck explorations are reduced.

Recurrent nerve damage should be a rare sequelae of a parathyroid neck exploration. In the current series no permanent vocal cord palsies occurred. In other series, this complication has been reported to occur in up to 10% of cases. The exposition of the nerve is unavoidable for the proper identification of at least the upper parathyroid gland. The unilateral neck exploration also implies less risk of hemorrhage. Should a sudden hemorrhage occur within the operation area after surgery the risk of a tracheal compression is greatly reduced if only one side of the neck has been explored. An important principle in the outlines of the presented surgical strategy is to complete the exploration of one side before exploring the other. The thorough search should be limited to the part of the neck area that is attainable from a collar incision. The removal of a thyroid lobe when one normal gland is missing is not advocated. The same is true for mediastinal

exploration, which should not be undertaken with less than preceding localization studies. Preferably, the microscopic examination of one whole macroscopically normal gland is used for the differentiation between uni- and multiglandular involvement. Still, the demonstration of a suppressed remnant containing fat-droplet rich chief cells in the adenoma-bearing gland is a good indication although not a proof of uniglandular disease. It is an obvious advantage if the pathologist already, during surgery, can demonstrate such a remnant as an indication of uniglandular disease. The removal of one whole macroscopically normal gland for diagnostic purposes should not be performed with less than the demonstration of at least one additional normal-looking gland if both sides have been explored. Hypocalcemia, early postoperative or late permanent, requiring vitamin D substitution may occur even when meticulous atraumatic technique is used. The varying anatomic position of the glands as well as their vascular supply make them vulnerable. With the increasing amount of parathyroid surgery the necessity to define a proper, safe, and atraumatic exploration method is obvious. In a survey of parathyroid surgery performed in the Scandinavian countries during one year, early hypocalcemia of about 10% could be demonstrated in patients operated for single adenoma (4).

Forty-three of the patients included in the current series were explored only at the adenoma side of the neck. S-calcium was normalized in these patients and remained so at the one-year follow-up. This fact indicates that parathyroid adenoma at the contralateral side cannot be present. Moreover, during the period in which the current consecutive series of patients was collected, no patients fulfilling the criteria of double adenoma were found. In a previous paper (2) we have defined the state of double adenoma as a situation in which two parathyroid glands are found harboring a typical chief cell adenoma and a remnant of normal chief cells showing suppression as indicated by abundance of intracellular fat droplets demonstrated by oil-red-O technique. In addition to these two glands, a whole macroscopically normal gland should be identified in which the parenchyma exhibits the normal histopathologic appearance of chief cells and, additionally, an abundance of intracellular fat droplets is demonstrable. In previous publications the frequency of double adenoma varies between two per cent and 30%

(5-11). It is important to realize that the concept of double adenoma must be based upon a histopathologic examination. Since the oil-red-O technique was applied in the routine intraoperative diagnosis of parathyroid disease, several cases of nodular hyperplasia have been found in which only two or three glands were macroscopically enlarged. The macroscopically normal glands in these cases exhibited invariably the same histopathologic picture of nodular hyperplasia. In about 250 consecutive patients in which the oil-red-O staining had been used, we have not encountered any case with one completely normal (i.e. functionally suppressed) gland besides two abnormal ones.

If, at all, a state such as double parathyroid adenoma exists, it should be unusual, probably with a frequency of less than one per cent. If the proposed surgical strategy is applied, the hypothetical patient with double adenoma could have them localized either both on the same side or one on each side. The likelihood for each alternative is 50%. In the case of a one-sided location of double adenoma, the proposed surgical strategy will disclose this unusual situation. Thus, it is only in one-half of the hypothetical cases of double adenoma that a contralateral adenoma might be missed. Still, should that be the case, a reoperation would be necessitated. This will then be performed in a previously unexplored region, making the operation equal to a primary exploration.

CONCLUSIONS

The application of unilateral parathyroidectomy as a routine procedure in primary hyperparathyroidism due to solitary adenoma is strongly advocated. A necessary prerequisite for its use is the intraoperative examination with oil-red-O technique of the microscopical specimens. With this method a functional dimension of the histopathologic examination is added to the morphologic. The removal of one whole, macroscopically normal parathyroid gland markedly increases the safety in the differential diagnosis between uni- and multiglandular disease. With the improved intraoperative diagnosis provided by oil-red-O technique it is not necessary to explore all four glands in order to exclude a multiglandular involvement.

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*IV. On the surgical strategy
in non-familial primary parathyroid hyperplasia
Long-term follow-up of 39 cases*

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ABSTRACT

Extent and result of surgery in 39 cases of nonfamilial primary parathyroid hyperplasia followed from 2 to 20 years are reported. Thirteen patients had been subjected to subtotal parathyroidectomy leaving no gland intact, while 26 had had less extensive surgery leaving at least one grossly normal or near normal gland intact without biopsy. In the former group, two patients (15%) developed permanent hypoparathyroidism requiring vitamin D treatment. In the latter group there were two patients (8%) with persisting hypercalcemia, which might have been avoided with a subtotal parathyroidectomy. Judging from these results, we believe that an individualized surgical approach is justifiable in nonfamilial primary parathyroid hyperplasia.

Subtotal parathyroidectomy, leaving no gland intact, is advocated as the method of choice only when all four glands are enlarged. If one or more glands are grossly normal or near normal, factors such as degree of hypercalcemia, symptoms, age, general condition and life expectancy should be taken into consideration when deciding the extent of the operation. A more conservative surgery leaving at least one grossly normal gland intact without biopsy appears to be sufficient for cure in most of these cases and minimizes the risk for development of permanent hypoparathyroidism.

INTRODUCTION

Subtotal parathyroidectomy with removal of all parathyroid tissue except for a part of one gland is generally regarded as the treatment of choice in primary parathyroid hyperplasia (5,9). As a variant of this procedure, total parathyroidectomy and autotransplantation has been advocated (10). These radical approaches are successful in most cases as regards elimination of hypercalcemia, but unfortunately they carry a risk for development of hypoparathyroidism requiring life-long medical treatment. The risk may seem to be negligible judging from the results reported in many studies on this subject. It should be kept in mind, however, that these good results usually emanate from very experienced endocrine surgeons, and the risk figure is different when it comes to parathyroid surgery in general outside specialized centers.

The amount of parathyroid tissue that may be left without persistence or recurrence of disease obviously varies - for unknown reasons - and in our experience, as well as others (1-3,8), it appears that a routine use of the methods mentioned above is to employ more force than is necessary in some patients with parathyroid hyperplasia. Insufficient follow-up and/or uncertain diagnostic criteria are the objections usually raised to question the validity of this experience. Thus, we think it is of interest to report our results from a comparatively conservative individualized approach in cases of nonfamilial primary parathyroid hyperplasia based on a histopathologically well defined material with a long-term follow-up.

MATERIAL AND METHODS

The histopathologic records of all surgical specimens registered as any kind of parathyroid lesion during a 21-year period (1960-1980) were reviewed. The microscopic slides were re-examined in all cases with descriptions indicating that at least two completely removed glands were abnormal. When only hematoxylin-eosin stained sections were available an unequivocal enlargement of two glands were required as a criterion for hyperplasia besides compact and nodular histologic structure. Enlargement of a gland was defined as weight exceeding 70 mg or, if the weight was not available, a section area larger than 50 mm^2 on the microscopic slide. When sections stained with modified oil-red-O (7) were available smaller glands

were accepted as hyperplastic if they contained compact areas or nodules of chief cells devoid of intracytoplasmic fat droplets indicating abnormal hyperactivity in a state of hypercalcemia (7). Nodules of oxyphil cells were not taken into account since such nodules are common in normal glands of elderly people and, furthermore, oxyphil cells normally contain small amounts of fat (7). In each of the fat-stained cases at least two glands weighed more than 65 mg and all glands showed a nodular and compact structure as well as signs of hyperactivity. It should be underlined that we agree with the opinion that hyperplasia involves all of the glands but may affect them very asymmetrically and far from always appear as a "four gland disease" macroscopically (4). When considering the wide variation in size of normal glands (10-70 mg) it is obvious that a gland within normal limits of size may be hyperplastic if the process starts in a gland at the lower end of the normal variation.

Cases of secondary hyperparathyroidism were excluded by an evaluation of the renal function at the time of operation. Patients with impaired renal function were accepted only if serum phosphate was low or normal. Patients with a history of familial parathyroid disease or hyperparathyroidism as part of a multiple endocrine neoplasia syndrome were excluded from the study.

Applying these criteria, we found 46 cases of surgically treated nonfamilial primary parathyroid hyperplasia. These cases represented 11% of all parathyroid operations performed during the sampling period. In three patients a total parathyroidectomy (without autotransplantation) had been performed deliberately. These patients were excluded. Another three patients had died within six months after the operation (two from cardiovascular disease and one from carcinoma). One patient refused to participate in any follow-up examination. Data regarding the latter four patients are presented separately in Table I.

Table I

Extent and result of operation in four patients with nonfamilial primary parathyroid hyperplasia followed less than two years

No. of glands removed	No. of glands biopsied	Observation time	S-Calcium at follow-up
2	-	1 month	normal
2	1	2 weeks	normal
2	1	6 months	normal
3	1	2 months	elevated*

* persisting hypercalcemia after reoperation with total parathyroidectomy. Autopsy refused.

The remaining 39 patients with an observation time of at least two years form the basis of this report. They could all be traced for follow-up. Values of serum calcium controlled within ten months prior to our follow-up were available for all patients still being alive. As regards deceased patients an examination of serum calcium made less than six months prior to the time of death was available in every case. The time from operation to the latest examination of serum calcium available constitutes what is here called "observation time". The longest observation time was 20 years and the mean observation time for the material as a whole 5.5 years (median 4 years). In patients classified as cured, the highest as well as the latest postoperative value of serum calcium was normal.

Twenty-six patients were females and 13 males. The age of the patients at the time of operation varied from 16 to 84 years with a median of 64. None of the patients had had any parathyroid operation before treatment at the Department of Surgery at Malmö General Hospital. Two patients had been subjected to previous thyroid operations elsewhere.

Thirty-five patients had chief cell hyperplasia and four had water-clear cell hyperplasia. In 20 cases histologic sections stained for fat with modified oil-red-O (7) were available.

The surgical procedures performed are listed in Table II. At surgery, exploration of four glands was always aimed at when more than only one gland was found to be abnormal. The finding of four glands was microscopically verified in 13 cases. Out of a total of 26 cases, in which at least one gland was left intact, less than four glands were found at operation in nine cases, and biopsy of grossly normal or near normal glands was deliberately refrained from in 17 cases. The reason for avoiding biopsy was, besides the size of the glands, factors such as mild hypercalcemia, lack of symptoms and advanced age or complicating disease making a troublesome hypoparathyroidism especially unfortunate.

Table II

Extent and result of operation in 39 patients with nonfamilial primary parathyroid hyperplasia followed for at least 2 years

No of glands removed	No of glands biopsied	No of patients	Mean observation time (years)	Result of operation		
				Permanent hypopara.	Normo-calcemia	Persisting hyperpara.
<i>GROUP I (At least one gland left intact)</i>						
2	-	7	7	1	6	-
2	1	2	7	-	2	-
3	-	17	5	-	13	4*
<i>GROUP II (No gland left intact)</i>						
2	2	7	5	1	6	-
3	1	6	5	1	5	-

*) See text for comment on findings at reoperation

Preoperative serum calcium ranged from 2.7 to 5.0 mmol/l with a median of 2.9 mmol/l (normal 2.2 - 2.6 mmol/l). Individual values for serum calcium with regard to the extent of operation are given in Figure 1. In Figure 2 preoperative serum calcium has been plotted against the total weight of three glands removed in ten cases where the fourth gland was identified and considered to be normal-sized.

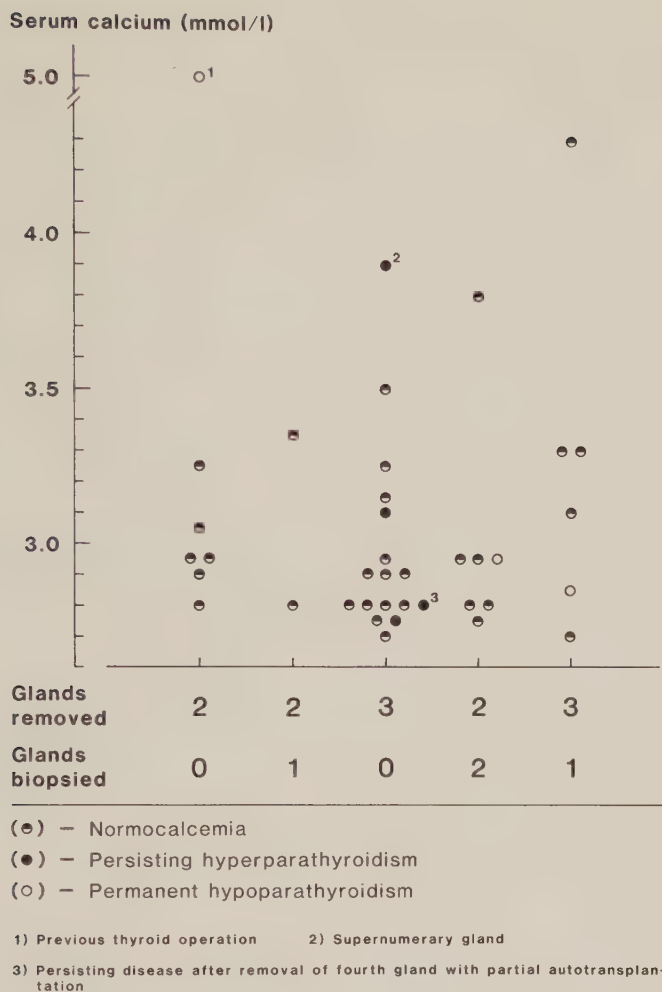


Fig 1. Preoperative serum calcium, surgical procedure and result of operation in 39 cases of nonfamilial primary parathyroid hyperplasia.

Serum calcium (mmol/l)

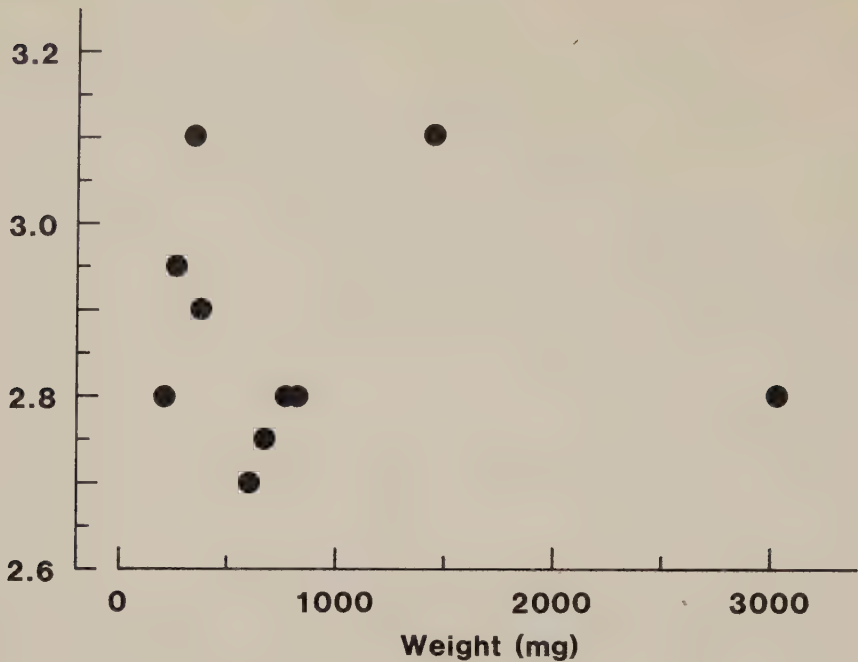


Fig 2. Preoperative serum calcium plotted against the total weight of three glands removed in 10 patients with a normal-sized fourth gland. Note the lack of correlation between degree of hypercalcemia and the size of the glands.

RESULTS

Thirty-two of our 39 patients with nonfamilial primary parathyroid hyperplasia were cured and have remained normocalcemic without supplementary therapy. These patients include three with waterclear cell hyperplasia, two of which had had subtotal parathyroidectomy and one removal of two glands only. Out of 29 patients with chief cell hyperplasia, nine had had subtotal parathyroidectomy leaving no gland quite intact, while less extensive operations had been performed in 20. The surgical procedures are listed in detail in Table II.

Persisting hypercalcemia occurred in four patients, all of whom had had three glands removed and one grossly normal gland left intact without biopsy. One of them still has persisting hypercalcemia after reoperation with removal of the fourth gland and autotrans-

plantation in the forearm of half of it. A second patient is normocalcemic after the same type of reoperation. In the third case of persisting hypercalcemia (with waterclear cell hyperplasia) a supernumerary enlarged fifth gland was found behind the oesophagus at reoperation. The enlarged gland was removed, still leaving one grossly normal gland intact. This patient is normocalcemic eight years after the second operation. The fourth patient has a borderline hypercalcemia three years after the operation (2.7 mmol/l compared to 3.1 preoperatively; upper limit of normal range 2.6 mmol/l). This patient, being 86 years old and asymptomatic, is not considered to be a candidate for reoperation.

Recurrent hyperparathyroidism after temporary postoperative normocalcemia has not appeared in this material so far.

Permanent hypocalcemia occurred in three patients. The surgical procedures performed in these cases appear from Table II. The patient with only two glands removed had been subjected to a hemithyroidectomy some years before the parathyroid operation. The latter was an emergency due to hypercalcemic crisis. Besides the two removed enlarged glands, one from each side of the neck, no other parathyroid tissue was identified at either operation. In the two other cases - with four glands identified - the excised glands were enlarged while partial biopsies were taken from glands considered to be normal in size (one and two respectively).

At the time of our follow-up 11 patients were dead. The cause of death was cardiovascular disease in five, carcinoma in four, infectious disease in one, and uremia in one. The latter patient had pyelonephritis and showed renal insufficiency already at the time of operation. The operation resulted in lasting normocalcemia, which was also the case in the other deceased patients.

DISCUSSION

As regards the extent of surgery, the material in this study may be divided in two major groups. One "conservatively" treated group comprises 26 patients in whom at least one parathyroid gland considered to be grossly normal or near normal was left intact without biopsy (group I). In the other group, consisting of 13

patients, no parathyroid gland was completely spared irrespective of size (group II).

In group I four patients showed persisting hypercalcemia. Two of these four cases should not have been prevented with a "three and a half gland" resection or total parathyroidectomy with autotransplantation. One of these patients is still hypercalcemic after reoperation at which the latter procedure was performed. The other patient was cured after removal of an abnormally located supernumerary gland. Thus, two treatment failures (8%) remain, one of which is asymptomatic and has a marginally elevated serum calcium. The cure rate in this "conservatively" treated group is hardly an overestimation due to erroneous diagnoses considering our hard criteria for selecting the cases. These hard criteria are reflected by the fact that our material constitutes a comparatively small proportion of the total number of parathyroid operations from which it emanates.

In group II, no patient had persisting hypercalcemia but two patients (15%) developed permanent hypoparathyroidism requiring treatment with vitamin D. We think this is a rather high cost for the benefit of minimizing the risk of persisting or recurrent disease which may be corrected with a second operation. This is especially worth considering if the patient is old and asymptomatic with a mild degree of hypercalcemia. This point has previously been stressed by Scholz and co-workers (8) in a paper based on a material similar to the present, and we agree with their statement that "it is preferable to err on the side of caution at operation and to accept a small risk of recurrence rather than to incur the chance of permanent hypoparathyroidism".

A "conservative" approach, in the sense that only enlarged glands should be removed, has been recommended by several authors in recent years (1-3). These authors, however, stress that biopsies of grossly normal glands must be done. Judging from the results presented here, we disagree with this statement. Biopsy from a grossly normal gland in a patient with established hyperplasia does not contribute to the diagnosis but unquestionably increases the risk of inducing hypoparathyroidism (6) and should not be done but for identification of parathyroid tissue if the presence of a

presumed gland is in doubt. In fact this is the only relevant purpose with an incisional biopsy in parathyroid diagnostics. For the distinction between a solitary adenoma and hyperplasia - not being obvious by the naked eye - the pathologist should get two complete glands. The reason is that the degree of hyperplasia may vary considerably not only from one gland to the other but also between different parts of one and the same gland. Sometimes the gland is only partially involved by the process. Thus, a normal-looking incisional biopsy does not exclude hyperplastic disease since it may not be representative for the gland as a whole.

As was underlined above, we agree with the conception that hyperplasia involves all glands at least microscopically and functionally although not always macroscopically (4). In our experience no gland is without microscopic evidence of hyperfunction in hyperplasia, which means that the diagnosis can be established or excluded by examination of two complete glands provided that parenchymal function is assessed besides glandular structure. This opinion is based on personal study of more than 250 continuously followed surgical cases of hyperparathyroidism treated during a 5-year period and examined by modified oil-red-O (7) parallel with conventional staining. Since the microscopic distinction between normal and slightly hyperplastic glands can be difficult or even impossible when based on structure alone - which varies with age as well as body constitution - we advocate staining of frozen sections with modified oil-red-O as an adjunct that adds information about parenchymal function to the interpretation of the structure (7).

Judging from the results presented here we believe that an individualized surgical approach is justifiable in non-familial primary parathyroid hyperplasia. Subtotal parathyroidectomy leaving no gland intact is the method of choice only when all four glands are enlarged. If one or more glands appear grossly normal or near normal, factors such as degree of hypercalcemia, symptoms, age, general condition and life expectancy should be taken into consideration when deciding the extent of the operation. A more conservative surgery leaving at least one grossly normal gland intact without biopsy appears to be sufficient for cure in most of these cases and minimizes the risk for development of permanent hypoparathyroidism.

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*V. Chronic parathyroiditis associated
with parathyroid hyperplasia and hyperparathyroidism*

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ABSTRACT

Two cases of chronic parathyroiditis associated with parathyroid hyperplasia and hyperparathyroidism are described. There was no evidence of an underlying infectious disease, a developmental anomaly, or a drug reaction that could explain the inflammatory component. It is suggested that an autoimmune process might have been involved in the pathogenesis of this previously unreported clinicopathologic entity.

INTRODUCTION

A case of hyperplastic parathyroiditis was recently reported (2). No biochemical measure of parathyroid function was available in that case, but the lesion was interpreted as an equivalent to Hashimoto's disease of the thyroid and hypofunction was considered to be a conceivable manifestation. We have encountered two cases of hyperplastic parathyroiditis both of which, however, presented clinically as primary hyperparathyroidism. We report herein our clinicopathologic findings.

CASE REPORTS

Case 1

A previously healthy 62-year-old man was operated on in 1977 because of suspected primary hyperparathyroidism, since hypercalcemia had been revealed at the time of treatment for kidney stones. Serum calcium was repeatedly increased to 2.8 mmol/liter (11.2 mg/dl) (normal range 2.2 - 2.6 mmol/liter = 8.8 - 10.4 mg/dl). Phosphate and creatinine in serum were normal. At operation four enlarged parathyroid glands were identified. The thyroid had a normal appearance. The three largest parathyroid glands were removed while the smallest one was left intact. The patient became normocalcemic the day after operation, and serum calcium was still normal at follow-up 4 years later.

The removed parathyroid glands weighed 1,580, 800, and 640 mg. They were partially composed of compact nodules of parenchyma split up by a dense hyaline fibrous stroma. The nodules were formed by a mixture of chief cells and oxyphil cells in varying proportions. The chief cells were devoid of intracytoplasmic fat droplets in frozen sections stained with oil red O. Within the parenchyma there were scattered dense accumulations of lymphocytes containing lymphoid follicles with germinal centers (Fig 1). Besides compact nodules, the glands showed areas with thin cords of parenchyma embedded in abundant loose fibrous stroma, which was diffusely infiltrated by numerous plasma cells and lymphocytes (Fig 2).

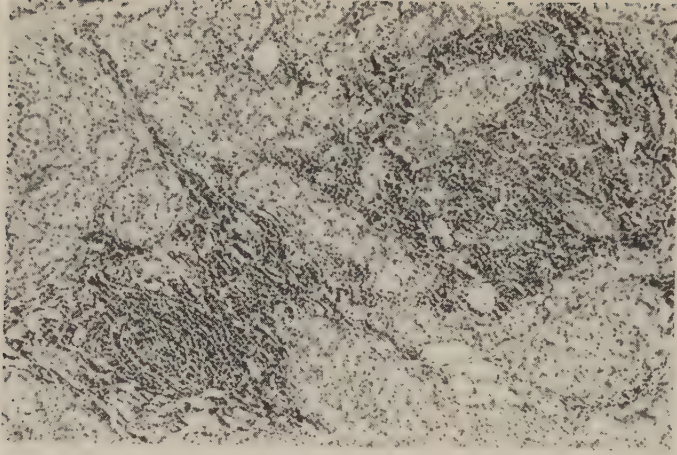


Fig 1. Compact nodules of parenchyma containing dense aggregates of lymphocytes (case 1). Note the formation of a lymphoid follicle.

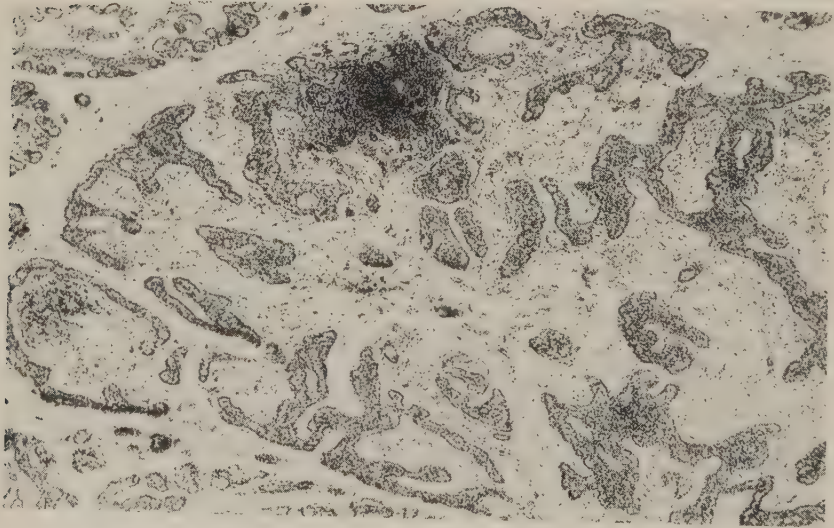


Fig 2. Area in case 1 with cords of parenchyma in abundant loose fibrous stroma. Note the numerous scattered plasma cells and lymphocytes throughout the stroma in addition to the large lymphoid follicle.

Case 2

A 53-year-old man was operated on in 1979 because of suspected primary hyperparathyroidism; hypercalcemia had been revealed at the time of treatment for kidney stones. Serum calcium was repeatedly increased to 2.7 mmol/liter (10.8 mg/dl). Phosphate and creatinine in serum were normal. Besides kidney stones the patient suffered from advanced ankylosing spondylitis. At operation four parathyroid glands were identified. The thyroid had a normal appearance. Three enlarged parathyroid glands were removed while the smallest one, considered to be normal-sized, was left intact. The patient became normocalcemic the day after operation, and serum calcium was still normal at follow-up 3 years later.

The largest removed parathyroid gland weighed 400 mg and the other two 100 mg each. Microscopically, they consisted of nodules and cords of parenchyma with a mixture of chief cells and oxyphil cells embedded in fibrous stroma.

The chief cells were largely devoid of intracytoplasmic fat droplets in frozen sections stained with oil red O. The fibrous stroma was partially dense and hyaline as well as partially loose and edematous. Numerous lymphocytes and plasma cells diffusely infiltrated the stroma and also were present as dense aggregates in the parenchyma with formation of lymphoid follicles (Figs 3-5). Signs of parenchymal destruction was evident in scattered areas (Fig. 6).

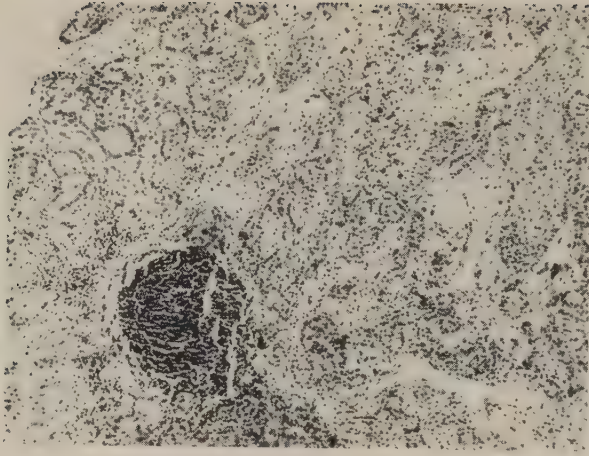


Fig 3. Lymphoid follicle and diffuse infiltration of plasma cells and lymphocytes involving the stroma as well as the parenchyma (case 2).

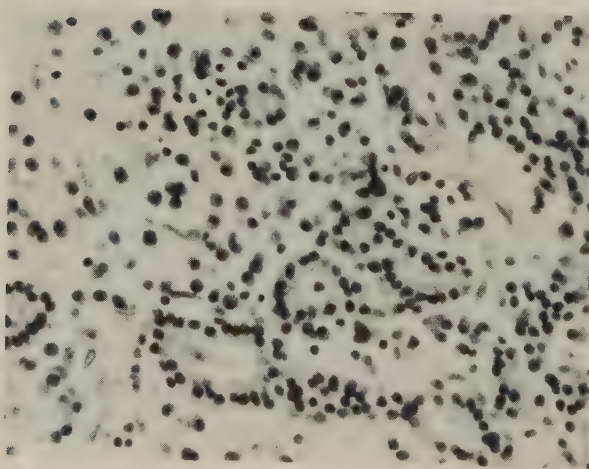


Fig 4. Detail from case 2 illustrating the abundance of plasma cells in the inflammatory process. Note the involvement of the parenchyma as well as the edematous stroma.

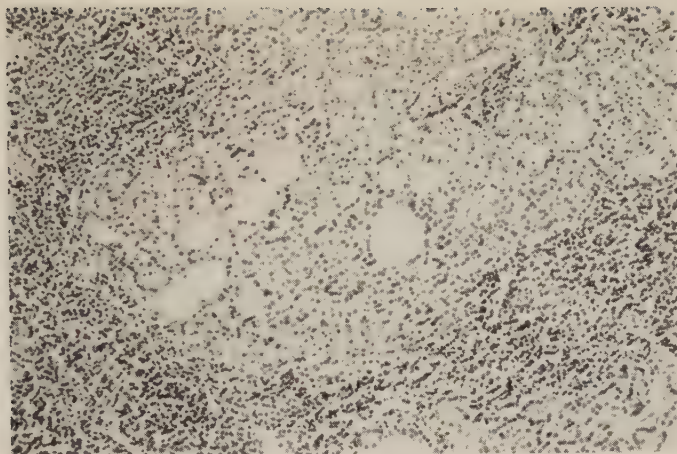


Fig 5. Area with massive infiltration of lymphocytes and plasma cells in case 2.

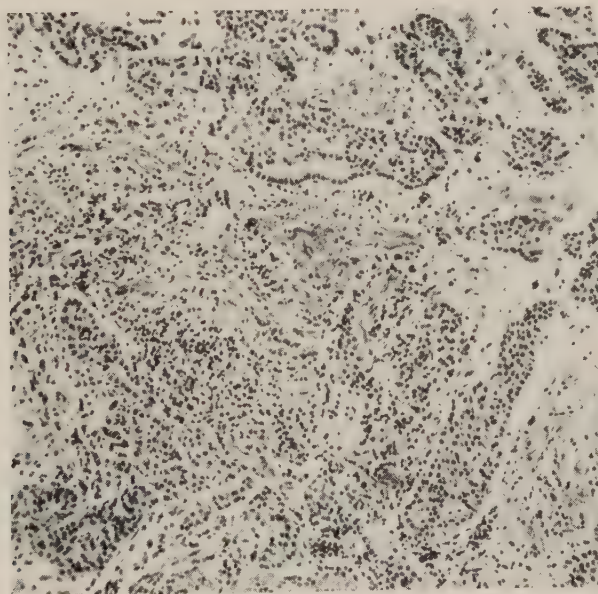


Fig 6. Signs of focal parenchymal damage in case 2. Note the accumulation of plasma cells and lymphocytes in association with disintegration of parenchyma and fibrosis in center.

DISCUSSION

The patients described here both presented clinically as cases of primary hyperparathyroidism, and histopathologically both showed parathyroid hyperplasia associated with chronic parathyroiditis. Minute focal aggregates of lymphocytes are rather common findings in the parathyroid glands at autopsy (7,8), especially in patients with severe infections, but a chronic inflammation as pronounced and widespread as described is rare. In two extensive studies of parathyroid glands from 800 autopsies only a single comparable case was found (8,9). Regarding functional disturbances, chronic parathyroiditis has been observed in a few cases of hypoparathyroidism (4,10), but as far as we know hyperparathyroidism associated with chronic parathyroiditis has not been reported before. The significance of the inflammatory component in the cases described here is uncertain and several possibilities must be taken into consideration.

Lymphocytic infiltration may occur in the parathyroid glands, among other tissues, as a nonspecific phenomenon in patients with infectious diseases. Such infiltrates are usually sparse and have a mainly perivascular localization (8) as distinct from the cases described here, which showed a marked diffuse infiltration of the stroma as well as involvement of the parenchyma with the occurrence of lymphoid follicles. Previous attempts to find a relationship between parathyroid disease and ankylosing spondylitis, which one of our patients suffered from, have failed (3). The other patient did not have any signs of disease apart from his hyperparathyroidism. Thus it seems most unlikely that the inflammation in these cases was a nonspecific parainfectious phenomenon.

Lymphoid tissue may occur in parathyroid glands as a result of incomplete separation from the thymus during the embryonal development. In our cases, however, the marked plasma cell infiltration and the occurrence of lymphoid follicles with germinal centers as well as the focal destruction of parenchyma are features not compatible with such an anomaly.

In our cases, the parathyroid glands contained scattered areas reminiscent of the picture described in rare, so-called parathyroid hamartomas, which may be associated with stromal inflammation as

well as hyperparathyroidism (5). Such hamartomas are, however, solitary lesions as distinct from our cases which showed multiglandular involvement.

The fact that a chronic parathyroiditis may be triggered in rabbits by exposure to ozone (1) raises the question of whether the parathyroiditis in our patients could have been a chemically induced disease, perhaps affecting already hyperplastic glands. This question has to be left open. In this context, it should be noted that the patients were exposed to different occupational environments, one being a road-worker and the other a consultant economist. Neither of them used any drugs.

Last, but not least, the possibility of an autoimmune process causing a chronic parathyroiditis also has to be considered. The clinicopathologic triad comprising glandular hyperplasia, hyperfunction, and lymphoid infiltration in our cases shows similarities to Graves' disease which is associated with immunologic factors acting as stimulants of the thyroid gland. The absence of intracytoplasmic fat droplets in the chief cells of all parathyroid glands examined in our patients indicates that the parenchyma was not only hyperplastic, but also hyperfunctioning (6), and thus that the hypercalcemia was not caused only by passive release of hormone from chief cells damaged by the inflammatory process. Unfortunately, our patients have not been accessible for any studies of possible immunologic abnormalities. Therefore, we can only suggest that an immunologic mechanism might have been involved in the pathogenesis of the parathyroid disease in these cases.

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